Review

Advances in space microbiology

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SUMMARY

Microbial research in space is being conducted for almost 50 years now. The closed system of the International Space Station (ISS) has acted as a microbial observatory for the past 10 years, conducting research on adaptation and survivability of microorganisms exposed to space conditions. This adaptation can be either beneficial or detrimental to crew members and spacecraft. Therefore, it becomes crucial to identify the impact of two primary stress conditions, namely, radiation and microgravity, on microbial life aboard the ISS. Elucidating the mechanistic basis of microbial adaptation to space conditions aids in the development of countermeasures against their potentially detrimental effects and allows us to harness their biotechnologically important properties. Several microbial processes have been studied, either in spaceflight or using devices that can simulate space conditions. However, at present, research is limited to only a few microorganisms, and extensive research on biotechnologically important microorganisms is required to make long-term space missions self-sustainable.

INTRODUCTION

The International Space Station (ISS) harbors a variety of microorganisms, originating as contaminants from Earth, components of experiments, or the normal microbiota of crew members (Castro et al., 2004; Checinska Sielaff et al., 2019; Pierson, 2001; Singh et al., 2018a; Venkateswaran et al., 2014; Voorhies et al., 2019). Research on microbial survivability upon exposure to space conditions began more than 50 years ago (Horneck et al., 2010; Taylor, 1974). Initial experiments involving exposure of microorganisms to spaceflight conditions started as early as 1935 with balloon flights and rocket payloads (Dickson, 1991; Nickerson et al., 2004). It was in 1966 that the National Aeronautics and Space Administration (NASA), during the Gemini IX and XII missions, began experiments in which bacteriophage T1 and spores of the fungi *Penicillium roqueforti* were directly exposed to space conditions for 16.8 and 6.5 h, respectively. However, they found that microbial survivability was almost negligible. This finding was later learned to be due to non-penetrating radiation in space, including solar UV radiation or soft X-rays, because covering the samples with a thin layer (0.4 mm) of aluminum resulted in 3,000-fold higher survival of bacteriophage T1 and nearly 100% survival of fungal spores (Hotchin et al., 1968). This study was one of the first to assess the survival limits of microorganisms upon exposure to space conditions.

Since the advent of manned space missions, several procedures, such as using freeze-dried food and preflight crew quarantine, have been implemented to monitor microbial population in the ISS (Castro et al., 2004, 2013; Rogers, 1986). However, exposure to spaceflight conditions and the immunocompromised state of the individuals might still result in otherwise commensal microorganisms becoming pathogenic (Klaus and Howard, 2006). It is well known that microorganisms undergo certain genetic and physiological changes to adapt to stress conditions, and the ISS, likewise, poses a stressful environment to biological life (Horneck et al., 2010). Several studies have been conducted to understand how microorganisms adapt to these space conditions, but these studies are limited to a few species and also require researchers to further understand changes at the genetic level (Nickerson et al., 2004). The microbial species that have been isolated from the ISS include both potentially pathogenic and industrially important microorganisms (Checinska Sielaff et al., 2019). Therefore, understanding how these microorganisms adapt to space conditions will aid in developing strategies to mitigate the risk posed by pathogenic microorganisms to the health of crew members, who might be in an immunocompromised state in the ISS (Lesnyak et al., 1996; Taylor et al., 1997). In addition, understanding how space conditions alter microbial processes in industrially important microorganisms will provide us information on how to engineer these microbes for efficient production of important compounds (Blachowicz et al., 2019b; Romsdahl et al., 2018, 2019). The knowledge gained from these studies will be of utmost importance in implementing safety measures for long-duration

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spaceflights and interplanetary explorations involving humans. This undertaking will also inform how physical forces influence microorganisms at the cellular, molecular, and evolutionary level, which can be translated to studies on Earth.

In this review, we first discuss the different extreme conditions that are experienced by microorganisms in space and how these conditions influence microbial processes. We also introduce some devices that have been developed to simulate and study the individual effect of microgravity or radiation on microbial processes. Finally, we discuss the applied aspects of space microbiology and how the advent of omics technology has contributed to the generation of data. In addition, we present some perspectives that warrant future investigation.

INFLUENCE OF EXTREME SPACE CONDITIONS ON MICROORGANISMS

The ISS has a stable orbit in the thermosphere of the Earth's upper atmosphere and circles the Earth at an average altitude of 400 km (Castro et al., 2013). The microbial species in outer space, that is, in the low earth orbit (LEO), thus, experience a plethora of stress conditions. These include altered gravity (10^{-3} to 10^{-6} g), temperature (153–393 K), space vacuum (pressure 10^{-7} to 10^{-4} Pa), and elevated CO_2 levels (partial pressure of 0.2–0.5 kPa) (Checinska Sielaff et al., 2019; Horneck et al., 2010; Huang et al., 2018; Senatore et al., 2018). In addition, LEO features galactic cosmic radiation (GCR), solar cosmic radiation (SCR), and a radiation belt trapped by the Earth's magnetosphere that can cause elements to ionize (Horneck et al., 2010). These radiations are composed of high-energy particles; for instance, GCR comprises high-energy protons (90%), α-particles (9%), and heavy particles (1%); SCR is primarily composed of protons and electrons, α-particles, and heavy particles (Ohnishi and Ohnishi, 2004; Senatore et al., 2018). In addition, the radiations trapped by Earth's magnetosphere consists of protons and electrons (Ohnishi and Ohnishi, 2004; Senatore et al., 2018). Most of the experiments on the ISS are conducted in a controlled environment within a space capsule, which is a pressurized module with life support systems where microorganisms are protected from most of the extreme conditions in space except for microgravity and cosmic radiations. Therefore, the differences observed in microbial physiology at the cellular or genetic levels are mostly attributed to the effect of microgravity and cosmic radiation (Senatore et al., 2018), unless the studies are conducted outside the space station (Horneck and Zell, 2012). Microgravity refers to the condition of weightlessness that occurs because of reduced physical force exerted by gravity, where gravity is not equal to zero but ranges from 10^{-3} to 10^{-6} g (Herranz et al., 2013; Huang et al., 2018; Nickerson et al., 2004). Convection currents are almost absent in microgravity, thereby resulting in low-shear and low-turbulence conditions; it is the alteration of these physical forces that affect microbial cell physiology (Hammond et al., 2000; Klaus et al., 1998). On the basis of results obtained from several experiments conducted under spaceflight conditions, it was hypothesized that the effect of microgravity on microbial cells is dependent on their motility (Benoit and Klaus, 2007). In the case of non-motile cells, a reduction in lag phase and an increase in cell density under spaceflight microgravity conditions were observed compared with ground controls (Klaus et al., 2004; Zea et al., 2017). This finding was attributed to the fact that the fluid around the non-motile cells remains quiescent, resulting in reduced mass transfer (nutrient uptake and release of metabolic byproducts) between the cell and the surrounding microenvironment. A result might be an altered chemical makeup surrounding the cell, eliciting a biological response. On the contrary, in motile cells, flagellar movement results in continuous mixing of the cell and surrounding environment, allowing improved mass transfer and counteracting the effect of microgravity or a low-shear environment (Thevenet et al., 1996). Thus, microgravity alters microbial responses indirectly because of the physical forces surrounding the cells that modify the extracellular environment of the cells, which in turn affects mass transfer between cells and their surrounding environment.

The influence of microgravity on the cellular properties of microorganisms appears to be multifaceted. For instance, simulated microgravity resulted in an increase in membrane fluidity in *Escherichia coli*, which could be responsible for increased drug resistance (Baker et al., 2004). However, results obtained for *Pseudomonas aeruginosa* showed no change in membrane fluidity under simulated microgravity (England et al., 2003). Some recent reports have focused on the effect of microgravity at the genetic level; for instance, expression of around 100 genes was altered in *Salmonella* under simulated microgravity (Wilson et al., 2002a). These gene products included transcription regulators, virulence factors, lipopolysaccharide synthesis enzymes, and iron utilization enzymes. Another study reported an increase in the rate of plasmid exchange under spaceflight conditions compared with ground controls in Gram-positive bacteria (DeBoever et al., 2007), implying that genetic exchange might play a role in adaptation to spaceflight stress conditions.

Further research is warranted to understand and correlate the specific genetic factors responsible for adaptation to microgravity conditions. The results from these studies can also be translated to infection by microbial pathogens in certain areas of the human body where they experience similar low-shear conditions, like microvilli of epithelial cells in the gastrointestinal, urogenital, and respiratory tracts of humans (Nickerson et al., 2004; Senatore et al., 2018).

Cosmic radiation is another important parameter that influences microbial processes in the ISS. Radiation results in biological effects via generating mutations either through direct energy absorption by biomolecules (proteins and nucleic acids) or indirectly through the interaction of biomolecules with radiation-induced radicals that are generated by different surrounding processes (Horneck et al., 2010; Huang et al., 2018). A Biostack experiment was performed with the spores of *Bacillus subtilis*, where a sandwich of monolayers of bacterial spores was mounted on cellulose nitrate foils and exposed to the outer space radiations in a spaceflight experiment (Bucker and Horneck, 1975; Horneck, 1993). The results obtained from this experiment led to the conclusion that the spores (1 µm diameter) on the outer areas were not affected by the high-energy particles of the galactic cosmic radiations; instead, a "bystander effect" was observed in which the effect of charged particles traversed from the outer layer of spores to the surrounding spores, where a biological effect was observed. Additionally, DNA is more prone to be damaged by radiation through the formation of double-stranded breaks that ultimately result in mutations, as observed in *E. coli*, *B. subtilis*, and *Deinococcus radiodurans* (Micke et al., 1994; Schafer et al., 1994; Zimmermann et al., 1994). Microorganisms possess several mechanisms to repair DNA, either by homologous recombination or non-homologous end joining (NHEJ). However, the ability to tolerate radiation depends on the extent to which the organism can repair DNA using DNA repair pathways; for example, *D. radiodurans* is about 5 times more resistant to ionizing radiation than *B. subtilis* spores (Baumstark-Khan and Facius, 2002; Senatore et al., 2018). The extent of this ability to repair DNA, mostly via NHEJ, results in mutations and is thus responsible for the scope of survivability of microorganisms in the presence of space radiations.

In addition to cosmic radiation, direct exposure to solar UV-A radiation and visible light in outer space can result in the production of reactive oxygen species and cause damage to nucleic acids, proteins, and lipids (Senatore et al., 2018). It can also result in the synthesis of photolyase, that is, a specific light-dependent repair enzyme (Senatore et al., 2018). The absorption of photons from solar UV radiation and its excitation results in the formation of bipyrimidine lesions in DNA. These lesions lead to the generation of cyclobutane pyrimidine dimers and pyrimidine (6-4) pyrimidone photoproducts between adjacent pyrimidine residues on the same DNA strand in vegetative cells (Cadet et al., 1992). Another type of bipyrimidine lesion, 5,6-dihydro-5(α-thyminyl) thymine, known as spore photoproduct (SP), was observed to be generated in bacterial spores. Formation of SP was likely due to the effects of spore desiccation, the presence of dipicolinic acid, and the binding of small, acid-soluble spore proteins to the DNA, in addition to the UV radiation (Nicholson et al., 2000). For instance, endospore monolayers of different *Bacillus* sp. exposed to simulated Mars-UV radiation exhibited differential inactivation kinetics, with *Bacillus pumilus* being most resistant, thereby indicating differences in their ability to activate repair pathways (Newcombe et al., 2005; Schuergera et al., 2006).

Similar to bacteria, many fungal species and their spores are highly resistant to radiation. In most fungi, the major pathway responsible for the repair of double-stranded breaks in DNA is NHEJ. Most double-stranded breaks are thus repaired without using a homologous DNA sequence, eventually resulting in mutations (Arentshorst et al., 2012). Interestingly, conidial monolayers of some fungi, when exposed to simulated Martian UV-C radiation, displayed enhanced UV resistance compared with *Bacillus* endospores (Blachowicz et al., 2019a). In another study, *Aspergillus* sp. and *Penicillium* sp. were found to dominate among different fungal species sampled from the ISS over a period of 6 years (Novikova et al., 2006). In addition to this component, some fungi, for example, *Saccharomyces cerevisiae*, exhibit large-scale genomic rearrangements under stress conditions that assist its survival (Chan et al., 2007). Thus, exposure to space radiation can result in an increase in mutation frequency throughout the microbial genome, thereby affecting several microbial processes.

GROUND SIMULATION OF SPACE MICROGRAVITY CONDITIONS

Spaceflight experiments are particularly challenging with respect to spacecraft and astronaut health. There are several limitations associated with conducting experiments on spacecraft, including concerns regarding crew health, limited flight opportunities, significant costs, the requirement of specialized

equipment to perform experiments, and limitations in power, work area mass, and crew time (Castro et al., 2013; National Academies of Sciences and Medicine, 2018; Nickerson et al., 2004). All these limitations restrict the range of experiments that can be conducted on spaceflight. Therefore, devices that can simulate space conditions on Earth are necessary to expand our understanding of the effects of space conditions on microorganisms. It is important to note that the ground-based simulators are not an exact representation of spaceflight conditions. However, they can still provide us with key information on how microorganisms adapt to these conditions (Baker and Leff, 2005; Baker et al., 2004; Benoit and Klaus, 2005; Huitema et al., 2002). Importantly, such devices can be used to conduct preliminary experiments before conducting the spaceflight experiments, which will be discussed later in this review. In addition, these simulator devices help to delineate the effect of individual conditions on the adaptation and evolution of microorganisms, rather than a combined effect of different conditions experienced during spaceflight.

Several ground-based systems are available to simulate space microgravity that can be selected based on the exposure required. In cases where short-duration exposure to microgravity, ranging from a few seconds to a few minutes, is required, space stations can be replaced with sounding rockets (3–13 min; 10^{-4}–10^{-3} g), parabolic flights (20–30 s; 10^{-3}–10^{-2} g), or drop towers (5 s; 10^{-5}–10^{-2} g) that provide free fall (Herranz et al., 2013; Huang et al., 2018; Senatore et al., 2018; Thomas et al., 2000). Long-duration exposure, on the other hand, requires special devices that can simulate microgravity conditions ranging from a few days to a few months (Castro et al., 2013; Herranz et al., 2013). Microgravity Simulation Support Facility (MSSF) at Kennedy Space Center (KSC) harbors such simulators that can be accommodated within controlled environmental chambers to conduct experiments, mimicking the controlled environment in the ISS (NASA document SP-2017-10-1096-KSC). Such ground-based simulators cannot be used to achieve real weightlessness because the magnitude of Earth's gravity cannot be changed. Nevertheless, they are still able to achieve "functional near-weightlessness" by changing the gravity vector continuously and constantly, with the gravity averaged to near zero (Herranz et al., 2013; Huang et al., 2018; Briegleb, 1992). These ground-based microgravity simulator devices (Figure 1) are described in the following sections.

Clinostats

A clinostat is a device that enables the rotation of a sample along one or two axes such that the cells remain in a constantly suspended state while continuously changing direction, therefore preventing the sample from perceiving the gravity vector (Huang et al., 2018; Klaus, 2001; Briegleb, 1992). A classical 2D clinostat was developed in 1879 by Julius Sachs, rotating along one axis, perpendicular to the gravity vector (Senatore et al., 2018). A 2D clinostat is now rarely used, and its derivative, a 3D clinostat, was later developed to improve the quality of simulations. A 3D clinostat uses two independent frames rotating along two different axes (Herranz et al., 2013), changing its orientation at a constant speed and direction relative to the gravity vector such that the sample remains in a suspended state by eliminating the effect of gravity (Huang et al., 2018). The 3D clinostats are appropriately called random positioning machines (RPMs), because the two frames can be rotated with different speeds and different directions, thus resulting in randomly changing speed and direction (Borst and van Loon, 2009; van Loon, 2007). A clinostat can thus be used to achieve a state of relative motionless of a cell with respect to its extracellular environment, as the cells remain in a suspended state. Therefore, 3D clinostats can be used to mimic the quiescent, unstirred fluid conditions characteristic of samples in orbit (Horneck et al., 2010). The use of RPMs has been found to be particularly suitable for studying the effect of simulated microgravity on higher organisms like plants, because they provide a suitable environment for 3D tissue morphogenesis (Hoson et al., 1997).

Rotating Wall Vessels (RWVs)

Rotating wall vessels (RWVs) consists of a hollow disk or cylinder that is completely filled with a liquid medium, without any bubbles, that rotates on one axis, perpendicular to the gravity vector. As opposed to clinostats, upon solid-body rotation, RWVs maintain the cells in a continuous fall state without being allowed to sediment at the bottom, thus achieving a low-shear, low-turbulence environment (Barrila et al., 2010; Horneck et al., 2010; Nickerson et al., 2004). This free-fall state allows the mass transfer and localized mixing with the extracellular environment, and air exchange via a gas-permeable membrane on one side (Nickerson et al., 2004). Various analogs of RWVs that are available include rotating wall bioreactors, rotating cell culture systems, and high-aspect rotating vessels. These RWV analogs function on the same
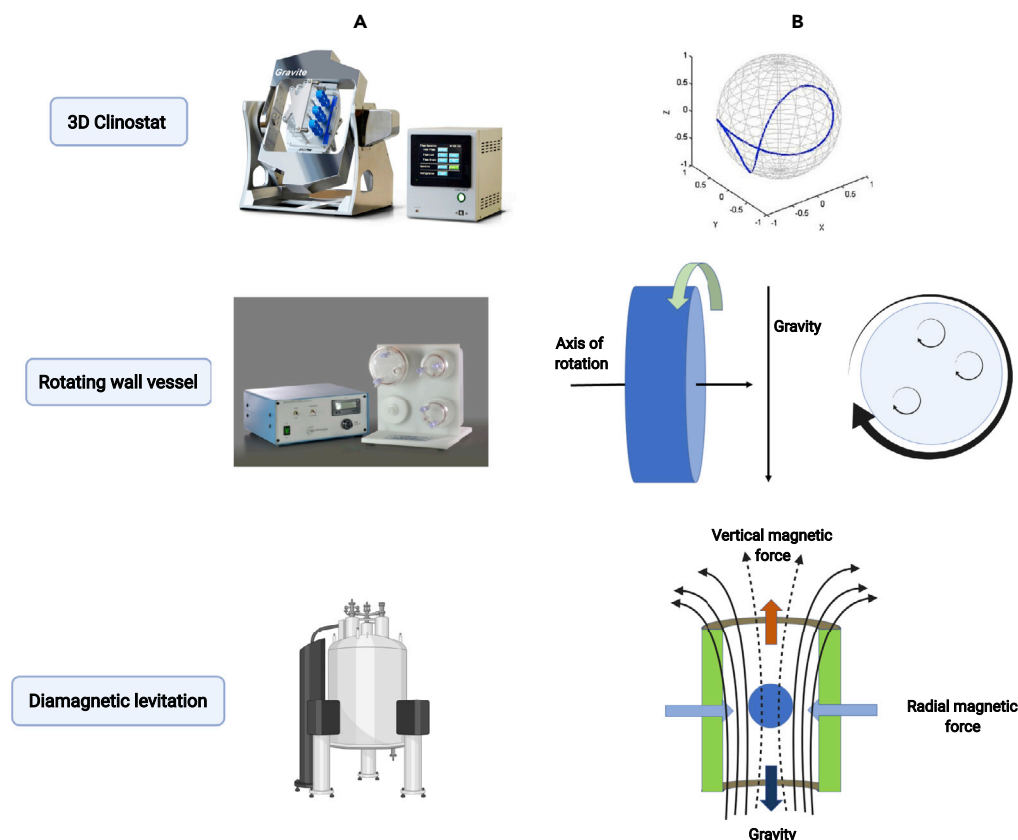
Figure 1. Ground-based microgravity simulators

(A and B) Different (A) ground-based simulators that are commonly used and (B) their mode of action to mimic space microgravity conditions. The figure has been generated using BioRender (https://biorender.com/). The source of images for 3D clinostat and rotating wall vessel is As One International, Inc. and Synthecon Inc., respectively. The image for the mode of action of 3D clinostat is adapted from elsewhere (Borst and van Loon, 2009).

principle of creating conditions of low-shear modeled microgravity (LSMMG), where cells remain in a free-fall state upon rotation, similar to space microgravity (Huang et al., 2018).

RWV was first developed by the NASA Johnson Space Center (Houston, Texas, USA) to study the effect of simulated microgravity on cell cultures, as it was shown that the low-shear environment supports the generation of 3D differentiated tissue-like assemblies (Freed et al., 1997; Freed and Vunjak-Novakovic, 1997; Unsworth and Lelkes, 1998; Vunjak-Novakovic et al., 2002). Thereafter, they have been used to study the impact of microgravity on different cell types, ranging from microorganisms to higher organisms (Crabbe et al., 2010; Fang et al., 2000; Fang et al., 1997a, b; Hammond and Hammond, 2001; Nickerson et al., 2000; Schwarz et al., 1992).

An important parameter to be considered while using clinostats or RWVs to maintain the cells in a continuous free-fall state is the rotation rate. A comparatively higher rotation speed will result in moving the cells outward near the wall of the container, and a lower rotation speed will result in sedimentation of the cells in a rolling motion on the bottom of the container (Klaus et al., 1998). A rotation speed higher or lower than the required speed will thus result in impairing the free-fall state or simulated microgravity effect. The changes in cell density and mass transfer between the cells and the extracellular environment should also be considered while defining the rotation speed (Begley and Kleis, 2002; Klaus et al., 1998).

Diamagnetic levitation

Diamagnetic levitation technology uses a strong, spatially varied magnetic field produced by a Bitter solenoid, or a superconducting solenoid magnet, to simulate near weightlessness. The diamagnetic force

opposes the gravitational force on a levitating object and thereby reduces internal stresses induced by the gravitational force to nearly zero (Herranz et al., 2013). This technology has been previously used for studying the effect of microgravity on many types of organisms, including protozoans, microorganisms, plants, and animals (Coleman et al., 2007; Guevorkian and Valles, 2006; Kuznetsov and Hasenstein, 1996; Liu et al., 2010). However, the major limitation to its use is that the magnetic field can itself influence the organism's physiology, in addition to microgravity (Liu et al., 2011). However, if the experiments are performed using appropriate controls for the influence of the magnetic field, it can still be practical.

GROUND SIMULATION OF SPACE RADIATION CONDITIONS

Another important parameter that influences microbial life in outer space is the high level of radiation. Although it is not feasible to simulate space radiation conditions on Earth because of the presence of multiple radiations with different energies in space, some devices with controlled radiation exposures have been developed to study their effect on microorganisms. Heavy-ion accelerators and polychromatic UV sources have been used to simulate cosmic rays and solar UV radiation, respectively (Horneck et al., 2010). For a long time, single ion beam accelerators with fixed energies were used to simulate space radiation conditions. However, its use was later limited because space radiation comprises a variety of ions with different energies (Kim et al., 2015; Norbury et al., 2016). In more recent times, this technology has developed substantially, such that it is now feasible to accelerate multiple beams with different energies, with beam switching times of less than 2 minutes. This technology has been implemented at the NASA Space Radiation Laboratory (NSRL) at the Brookhaven National Laboratory (BNL) (Kim et al., 2015; Norbury et al., 2016). Heavy-ion accelerators to simulate space radiation condition are also available at Deutsches Zentrum für Luft-und Raumfahrt Planetary and Space Simulation facilities (PSI) in Germany (Rabbow et al., 2012) and the National Institute of Radiological Sciences in Japan (Cortesao et al., 2020). Despite recent advancements, single-beam experiments are still important in understanding the effect of each radiation component and in deciphering whether the radiation components act additively or synergistically to produce an effect on a biological system (Norbury et al., 2016). These technologies allow one to simulate GCR and to study its effect on microorganisms and other biological life forms using ground-based facilities. Nevertheless, there are still some challenges associated with the use of these accelerators, such as determining the appropriate radiation dose, creating multiple experiments with multiple beams, and costs associated with the experiments (Norbury et al., 2016). Moreover, the use of a heavy-ion accelerator is not an exact representation of space radiation, but it can still be used to generate preliminary data before conducting spaceflight experiments.

APPLICATION OF MICROORGANISMS IN SPACE

Up to now, results from experiments conducted on spaceflight or using ground-based simulators described above have shown that microgravity conditions can alter microbial processes, including growth, morphology, gene expression, virulence, drug resistance, biofilm formation, and secondary metabolism (Baker et al., 2004; Crabbe et al., 2010; Demain and Fang, 2001; Kacena et al., 1999; Kim et al., 2013; Lawal et al., 2013; Van Mulders et al., 2011). A plausible explanation for these alterations is the loss of gravitational force, resulting in reduced mass transfer and thereby changing various cellular processes (Zea et al., 2016). It is important to study how these conditions alter the cellular processes for two main reasons. First, microorganisms that acquire or display increased virulence or drug resistance properties can pose a threat to the health of crew members, especially in the closed habitat of the ISS (Crabbe et al., 2010, 2013; Rosenzweig et al., 2010; Wilson et al., 2007). Second, microorganisms with altered secondary metabolism under spaceflight conditions can produce novel compounds or display increased production of known compounds that are pharmaceutically or industrially important (Benoit et al., 2006; Lam et al., 1998, 2002). Some of the aspects of microbial species that are altered under spaceflight or simulated microgravity conditions are detailed in the following sections (Figure 2).

Production of secondary metabolites

For decades, it has been known that microgravity can affect cellular processes and functions in microorganisms (Taylor, 1974). Alterations in the production of secondary metabolites under space conditions have proved particularly intriguing because of their biotechnological implications. In fact, several spaceflight and ground-simulated experiments have demonstrated enhanced production of pharmaceutically relevant secondary metabolites from microorganisms (Blachowicz et al., 2019a; Knox et al., 2016; Romsdahl et al., 2019). Some of these gene clusters synthesizing secondary metabolites are either silent or expressed at
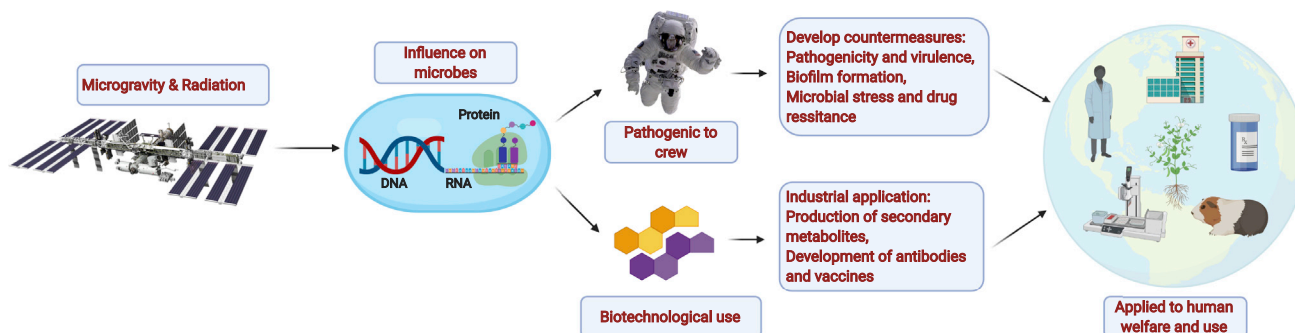
Figure 2. A brief overview of the need to study microbes on the ISS
The figure has been generated using BioRender (https://biorender.com/).

very low concentrations under normal, terrestrial conditions. Moreover, in space, cells can produce certain secondary metabolites in suspension and in the absence of shear forces (Klaus, 2004). Hence, spaceflight could offer unique advantages for bioprocessing applications.

Several studies indicated that secondary metabolism is likewise generally enriched in response to heightened extracellular environmental signals and stresses. For instance, *Humicola fuscoatra* samples aboard the US Space Shuttle mission STS-77 exhibited considerably higher production of the antibiotic monorden compared with ground controls (Lam et al., 1998). Specifically, the space samples displayed a 30% increase and a 190% increase in monorden production in T8 and PG media, respectively. Follow-up paired spaceflight and ground control experiments with *Streptomyces plicatus* samples showed an increased productivity of the antibiotic actinomycin D (Lam et al., 2002). After 17 days, comparisons of specific productivity in terms of the amount of antibiotic produced per viable cell showed a 296% increase and a 577% increase in defined and complex media, respectively. Similarly, several *Aspergillus* species exposed to spaceflight conditions or isolated from the ISS showed enhanced production of known or novel metabolites of economic importance. For instance, *Aspergillus fumigatus* isolates from the ISS exhibited increased production of the antibacterial compound fumigaclavine A compared with the reference strain (Knox et al., 2016). Subsequent genomic analyses revealed a distinct frameshift mutation in *fgaPT1* that likely accounts for the increased fumigaclavine A production. In another study, *Aspergillus nidulans nkuA* mutant exposed to spaceflight conditions resulted in significant production of asperthicin, an anthraquinone pigment (Romsdahl et al., 2019). *Aspergillus niger* strain isolated from the ISS exhibited a 6,000% increase in the production of an antioxidant pyranonigrin, compared with the reference strain ATCC 1015 (Romsdahl et al., 2020). Genomic analysis indicated the presence of INDELs within the promoter of *flbA*, a regulator of pyranonigrin production that might contribute to its enhanced production (Aerts et al., 2018). The induction of asperthicin synthesis in *A. nidulans*, and increased production of pyranonigrin in *A. niger*, can be explained as a response to provide protection to the fungi from space radiations. Collectively, these studies highlight the potential of space conditions to enhance the production of useful secondary metabolites.

Despite these promising results, other experiments suggest that microorganisms may respond to spaceflight and simulated microgravity conditions in diverse ways. For instance, the production of the immunosuppressive agent rapamycin by *Streptomyces hygroscopicus* was inhibited by simulated microgravity conditions (Fang et al., 2000). In fact, rapamycin production decreased by about 90% compared with normal gravity controls. Furthermore, longer-term studies have suggested that increased productivity by microorganisms in space conditions may be time-sensitive. Interestingly, Benoit et al. (2006) found that whereas increased productivity of actinomycin D by *S. plicatus* was observed on days 8 and 12, as reported previously (Lam et al., 2002), ground control productivity of actinomycin D surpassed that of spaceflight samples for the remainder of the mission (Benoit et al., 2006). Similarly, simulated microgravity experiments using RWVs showed increased production of the polyester polymer poly-b-hydroxybutyrate by *Cupriavidus metallidurans* after 24 h, but not after 48 h, compared with controls (De Gelder et al., 2009). These studies suggest that the effects of microgravity on secondary metabolism may be strain-, growth condition-, or pathway-specific and may be time-dependent. Therefore, additional studies in simulated microgravity conditions are needed to better characterize the microbial response to space conditions and, thus, maximize bioprocessing efficiency in this application.

Pathogenicity and virulence

The effect of space conditions on microorganisms spans beyond altered secondary metabolite production. Studies conducted with pathogens like *P. aeruginosa* (Crabbe et al., 2010), *A. fumigatus* (Knox et al., 2016), and *Fusarium oxysporum* (Urbaniak et al., 2019) reported that space conditions affect regulatory signals resulting in changes in gene expression profile and physiology, and as an extension pathogenicity. Enhanced pathogenicity and virulence of microorganisms in space could pose a significant risk to immunocompromised astronauts during long-term missions. Therefore the identification of microbial strains that can become pathogenic in spaceflight and gaining insight into the underlying mechanisms is crucial.

Owing to the increased duration and frequency of manned space missions, studies aimed at defining the pathogenicity and virulence of microorganisms in space have surged. In one of the studies conducted, *A. fumigatus* isolates from the ISS were shown to be significantly more lethal than clinical controls in a larval zebrafish model (Knox et al., 2016). Subsequent proteomic analyses revealed an increased abundance of proteins involved in the biosynthesis of trypacidin, a potent mycotoxin, in the ISS strains that could account for the increased virulence (Blachowicz et al., 2019b). Trypacidin has been previously reported to be cytotoxic toward alveolar lung cells (Gauthier et al., 2012). Therefore it becomes crucial to characterize the virulence of common microorganisms in space and understand its impact on crew health. Similarly, it was shown that fruit flies infected with the bacterial pathogen *Serratia marcescens* aboard the ISS displayed significantly shorter survival times than controls (Gilbert et al., 2020). The fact that this trend was upheld for strains grown on the ground in simulated microgravity conditions suggests that microgravity is the contributing virulence factor. In another study, *Salmonella enterica* serovar Typhimurium grown in LSMMG was found to be more virulent in a murine model of infection compared with its control culture, exhibiting an LD_{50} value 5.2 times lower than the strain grown under normal gravity (Nickerson et al., 2000). A follow-up study surprisingly showed a decrease in the expression levels of pathogenicity islands (SPI-1 and SPI-2) in the LSMMG-grown *S. enterica* culture, indicating a role of a previously unknown virulence mechanism or regulatory pathways (Wilson et al., 2002b). In the case of yeasts like *Candida albicans*, pathogenicity and virulence are known to be associated with its morphological transition from yeast to hyphal form. This transition has been reported in studies conducted with *C. albicans* in spaceflight or simulated microgravity (Altenburg et al., 2008). However, exposure of *C. albicans* to spaceflight conditions did now show an altered virulence compared with ground controls, as determined using an intra-peritoneal mouse model of infection. This result led to the conclusion that either short-term exposure to spaceflight did not significantly affect its virulence or the mode of infection was ineffective because intra-peritoneal is not the standard method for infecting *C. albicans* (Crabbe et al., 2013). Collectively, these studies exemplify the importance of defining microbial pathogenicity and virulence in space.

Biofilm formation

Several studies with bacterial and fungal species reported the formation of biofilms on spaceflight surfaces and its water systems, potentially corroding surfaces or becoming detrimental to crew health (Novikova, 2004; Ott et al., 2004; Schultz et al., 1989; Song and Leff, 2005). Microbial growth has been observed previously on different locations, including piping, water recycling system, electrical connectors, air-conditioning, and thermal control, aboard Mir space station (Klintworth et al., 1999). Among the 234 microbial species isolated from the Mir space station, most of the fungal species exhibited potential polymer biodegradation activity, thereby posing a threat to the structural integrity of the space station (Novikova, 2004). Several bacterial pathogens, mostly staphylococci and enterococci, have been shown to form biofilms on the surfaces of the ISS (Schiwon et al., 2013; Sobisch et al., 2019). Some bacterial species like *Bacillus* and coliforms that are present in wastewater or soil were isolated from water and waste lines in the ISS (Koenig and Pierson, 1997; La Duc et al., 2004; Ott et al., 2004). *Sphingomonas* sp. and *Methylobacterium* sp. were found to be dominant in potable water on the ISS (Novikova et al., 2006). Therefore, in the future, more emphasis should be given to materials or surface coatings that are resistant to biodegradation and biofilm formation (Zea et al., 2018).

The formation of biofilm under microgravity conditions was first reported by McLean et al. (2001); they showed that *P. aeruginosa*, when exposed to spaceflight conditions on polycarbonate filters, remained viable and formed a biofilm (McLean et al., 2001). Another report showed that *P. aeruginosa* formed a biofilm that was typical in structure, consisting of columns overlaid with a canopy under spaceflight conditions. Flagella-driven motility was found to be essential for this typical biofilm structure (Kim et al., 2013). Interestingly, it had been previously shown that a global regulator, Hfq, plays a role in *P. aeruginosa* to respond

to microgravity conditions, resulting in differential regulation of 167 genes and 28 proteins (Crabbe et al., 2010). In another study, *S. enterica* serovar Typhimurium exhibited increased cell clumping, aggregation, and production of an extracellular matrix, indicating increased biofilm formation under spaceflight conditions. The transcriptome and proteome analysis of *S. enterica* revealed the role of Hfq under spaceflight conditions, similar to that identified in *P. aeruginosa* (Wilson et al., 2007). Hfq is an RNA-binding protein that is conserved in both prokaryotes and eukaryotes (Valentin-Hansen et al., 2004). It is known to be important for regulating the expression of various genes involved in virulence and stress resistance in several opportunistic pathogens, by stabilizing small regulatory RNAs and interfering with their interaction with mRNA molecules (Ding et al., 2004; Sittka et al., 2007; Sonnleitner et al., 2003; Valentin-Hansen et al., 2004). Another study involving a different species, *Micrococcus luteus*, reported an increase in production of extracellular polysaccharides and enhanced biofilm formation under spaceflight conditions (Mauclaire and Egli, 2010). Further studies are warranted to understand the mechanistic basis of typical biofilm structures that are formed by other microbial species under microgravity conditions.

The ability of microbial species to form biofilms has also been demonstrated in LSMMG, similar to spaceflight. For instance, some bacterial species like *Klebsiella pneumoniae*, *P. aeruginosa*, *E. coli*, *Staphylococcus aureus*, and *Streptococcus mutans*, also showed increased aggregation and biofilm formation when grown under simulated microgravity or LSMMG conditions in RWV (Castro et al., 2011; Crabbe et al., 2010; Lynch et al., 2006; Orsini et al., 2017; Tucker et al., 2007; Wang et al., 2016, 2017). Thus far, studies on biofilm formation with lower eukaryotes have focused on only a few species. The experiments with the model yeast *S. cerevisiae* under simulated microgravity conditions showed that yeasts undergo random budding instead of bipolar budding, resulting in an increase in cell clumping (Purevdorj-Gage et al., 2006). A subsequent study conducted on *S. cerevisiae* in LSMMG showed that shear stress resulted in differential expression of genes involved in various cellular processes, including chromosome organization (Johanson et al., 2006). Further analysis of these findings will aid in understanding their role in the adaptation and survival of yeasts both under microgravity conditions and similar low-shear environments encountered in the human body. Another yeast, *C. albicans*, also showed a similar response of increased filamentation, cells undergoing random budding clustered with the filamentous forms, and changes in expression levels of two genes associated with the yeast-hyphae transition under LSMMG conditions (Altenburg et al., 2008; Searles et al., 2011). A subsequent report on *C. albicans* grown under spaceflight conditions further confirmed this finding (Crabbe et al., 2013). Microarray analysis indicated that random budding can be a consequence of altered expression of genes involved in the synthesis of the actin cytoskeleton and its polymerization because the actin cytoskeleton is essential for bud site selection (Crabbe et al., 2013; Ni and Snyder, 2001). These features might confer an advantage to virulent properties of *C. albicans* in long-term spaceflights in a species that is otherwise a human commensal.

Microbial stress and drug resistance

Several studies have indicated differential expression of stress response genes under spaceflight conditions, some of which result in altered drug susceptibility/resistance profiles in microorganisms. These microbial characteristics are of importance, particularly because of their impact on the health of crew members and the integrity of spacecraft. Some of the early studies conducted on *E. coli* and *S. aureus* exhibited increased resistance to all the antibiotics tested under spaceflight conditions when compared with ground controls (Lapchine et al., 1986; Tixador et al., 1985). Another experiment showed that this difference was observed only when cells were grown in liquid media and not when grown on agar, indicating a physical effect rather than a biological effect (Kacena and Todd, 1999). *S. typhimurium* and *E. coli* cells grown under simulated microgravity conditions showed enhanced resistance to acid stress, thermal stress, osmotic stress, and an increased ability to survive in macrophages, independent of the expression levels of global regulator RpoS. This finding indicates that the response is likely due to another stress response pathway (Lynch et al., 2004; Wilson et al., 2002a). In stationary-phase cells of *E. coli*, however, *rpoS* mRNA was found to be more stable under simulated microgravity, resulting in increased RpoS protein levels that might be responsible for enhanced stress resistance in the stationary phase (Lynch et al., 2004). In another study, *E. coli* were grown in the presence of increasing concentrations of the antibiotic gentamicin during spaceflight, which revealed that the cells adapted to the antibiotic on spaceflight earlier compared with ground controls. Transcriptome analysis showed that 50 stress response genes were upregulated in microgravity, including antibiotic stress-responsive genes (Aunins et al., 2018). Microbial cells present in a biofilm are often more resistant to antibiotics and other stresses, and this trend holds for spaceflight samples. For instance, *E. coli* biofilm exhibited increased resistance to salt stress, ethanol stress, and the antibiotics

penicillin and chloramphenicol under LSMMG conditions. Interestingly, the *E. coli rpoS* mutant showed impaired resistance to salt and ethanol stress but not antibiotic resistance, indicating the role of separate pathways for different stress conditions (Lynch et al., 2006).

In addition to mutations in the chromosome, some studies indicated the role of horizontal gene transfer in imparting drug resistance. A report showed that all Gram-positive bacteria were resistant to at least one antibiotic, the most abundant resistant element being the plasmid-borne erythromycin and tetracycline resistance genes that likely spread via horizontal gene transfer from staphylococci (Sobisch et al., 2019; Vaishampayan and Grohmann, 2019). *Bacillus thuringiensis* also exhibited an increased probability of transfer of genetic material (plasmids or chromosome fragments) in spaceflight conditions, which might explain its increased antibiotic resistance and virulence (Beuls et al., 2009; Senatore et al., 2018). In contrast to all these reports, some studies showed no difference in the antibiotic resistance profile postflight compared with the ground control, probably because of transient changes under spaceflight conditions (Juergensmeyer et al., 1999; Mora et al., 2019).

In the case of lower eukaryotic microorganisms, studies on responses to different stress conditions and antifungal drugs under spaceflight conditions are limited to only a few species. In a study on *S. cerevisiae* exposed to LSMMG, microarray analysis of the yeast revealed that among the genes differentially expressed, 26% of genes were involved in general environmental stress responses, whereas the remaining were unique to microgravity conditions (Sheehan et al., 2007). In another study on *C. albicans* exposed to spaceflight conditions, cells in the biofilm exhibited increased expression of ABC transporters and multidrug efflux proteins and downregulation of ergosterol-encoding genes, which are all involved in imparting drug resistance to the cells. In addition, several genes involved in oxidative stress resistance were also differentially regulated under spaceflight conditions (Crabbe et al., 2013). Among the Lsm family of proteins that are orthologs of Hfq in eukaryotes, expression of Lsm2 was significantly affected under spaceflight conditions. However, its role in the spaceflight response is yet to be determined (Crabbe et al., 2013). Similarly, *C. albicans* cells exposed to the LSMMG condition formed a complex biofilm structure and exhibited significantly more resistance to amphotericin B compared with ground control (Altenburg et al., 2008). Further research is needed to understand the physiological or genetic basis of drug resistance in human pathogens that might vary among different species.

SPACE CONDITIONS INFLUENCE ASTRONAUT MICROBIOME

So far, this review discusses the effect of space or simulated-space conditions on microorganisms and their impact on spacecraft and human health. However, in addition to being present in the space environment, microbes are an essential constituent of the human body that is also affected by space conditions. Studies on the effect of space conditions on human microflora have been ongoing since the Skylab mission. During the Skylab mission, although the microbial counts in the gastrointestinal (GI) samples of astronauts increased after the spaceflight, there was a reduction in microbial diversity (Taylor et al., 1971). The study also concluded an increase in pathogenic species, including *S. aureus* and *S. marcescens*, with *S. aureus* capable of being transferred from astronaut to astronaut. Later on, several culture-based studies showed that the space conditions result in alteration of the astronauts' oral, nasal, and GI microbial composition (Brown et al., 1976; Decelle and Taylor, 1976; Lencner et al., 1984). One of the studies utilized culture-independent methods to analyze the changes in microbial communities of GI tract, skin, nose, and tongue of astronauts during long-term space missions (Voorhies et al., 2019). The results showed that during 6 months flight, there was an increase in GI microbial diversity in four of five astronauts under observation; however, intestinal microbiota's composition became similar across astronauts with time in space. A slight increase in the inflammatory immune response during spaceflight was correlated to the altered GI microbiome in astronauts. In addition to GI, although the skin microbiome varied between different astronauts, common microbial shifts were observed among them during spaceflight. Crew members have reported allergies because they are continuously exposed to dust in a closed system of the ISS. Several human-associated bacterial commensals and a few opportunistic fungal pathogens were isolated from the ISS debris, which might be responsible for causing allergies (Venkateswaran et al., 2014). A different study also reported a higher abundance of *Malassezia*, lipophilic skin fungi, compared with other fungal species from astronauts' skin surfaces, during their 6 months stay on the ISS (Sugita et al., 2016). Another report showed differences in the gut microbiota of astronauts and humans on the earth, with astronauts exhibiting a significant decrease in microbial composition, mostly attributed to differences in their lifestyle and diet (Hao et al., 2018). Significant differences between an astronaut's gut microbiome during 1-year mission aboard the

ISS, compared with his twin on Earth, were also observed (Garrett-Bakelman et al., 2019). The human gut microbiome can have an effect on general physiological responses, immunity, and health of the body (Heyde and Ruder, 2015; Siddiqui et al., 2021). Therefore, it becomes crucial to maintain a healthy microbiome, especially in astronauts who are immunocompromised in space.

THE ADVENT OF OMICS TECHNOLOGY IN SPACE

Our understanding of microbial diversity in the upper atmosphere and aboard spacecraft has relied mostly on the culturable microorganisms. In recent times, the advent of genomics has allowed for the identification of new species (Bijlani et al., 2021; Singh et al., 2019; Venkateswaran et al., 2017) and several other microorganisms found in the ISS that are otherwise unculturable. It is well known that only 0.1%–1% of microorganisms from any environment are culturable (Lloyd et al., 2018). In a study conducted by Singh et al., metagenomic data generated from the ISS samples were used to retrieve nearly complete whole-genome sequences (WGSs) of bacterial species that led to the identification of a novel genus. The metagenome-assembled genome sequences of the novel species were shown to be identical to the WGS obtained from previously isolated ISS cultures. Thus, metagenomic data can provide nearly complete WGS that can be used to accurately identify novel species. This study was the first wherein the approach known as "metagenome to phenome" was used on the ISS samples and resulted in the identification of a novel genus (Singh et al., 2019). A different study developed Growth Rate InDex (GRiD) to determine the growth rates of ultra-low coverage or *de novo*-assembled metagenomes (Emiola and Oh, 2018). This represents an important parameter that can be applied to the metagenomics data generated from the ISS samples to determine the growth rate of different bacterial species, especially those that are lower in abundance. In the future, this approach can be extended to identify unculturable species as well.

The use of multi-omics, including genomics, transcriptomics, proteomics, and metabolomics, has particularly aided in studying the variations in microbial diversity aboard spaceflight. Several studies have utilized amplicon and shot-gun sequencing to study changes in microbial diversity at different time-points in different areas aboard the ISS (Checinska et al., 2015; Checinska Sielaff et al., 2019; Ichijo et al., 2016; Lang et al., 2017; Mora et al., 2016, 2019). In all the studies conducted, microbial diversity was found to vary at different time points aboard the spaceflight. The results showed that the ISS is chiefly composed of human-associated microbiomes, including Biosafety Level-2 (BSL-2) opportunistic pathogens. These opportunistic pathogens isolated from the ISS exhibited antibiotic resistance, but it was not found significant to result in an increase in pathogenicity under space conditions; however, the presence of most of the microorganisms indicated a risk toward damage to the integrity of spaceflight material (Mora et al., 2016, 2019). Further experiments are warranted to understand their antibiotic resistance profile and pathogenicity while aboard the ISS. A more recent study utilized metagenomics, in combination with a technique to analyze only viable cells on the ISS, to study the spacecraft's active microbial diversity (Singh et al., 2018b). The study led to the similar conclusion that microbial diversity varied at different time points at different locations aboard the ISS. The data indicated that BSL-2 microorganisms, for instance, *Klebsiella pneumonia*, persisted in the ISS over different time points. The metagenome-based analysis provides an advantage of higher sequence coverage and improved resolution compared with amplicon sequencing. Metagenomic sequences also helped in predicting that there was an increase in the virulence factors and antimicrobial resistance genes associated with the microorganisms over a time period in the ISS, which needs to be further validated when compared with appropriate ground controls (Singh et al., 2018b).

Recently, nanopore sequencing technology MinION, along with streamlined sample preparation protocol, was first tested during a parabolic flight, which was also followed by data analysis (McIntyre et al., 2016). This allowed researchers to understand the effect of microgravity on sequencing protocol and tools, and to further modify the procedure to implement on the ISS. Thereafter, MinION DNA sequencer was successfully established in-flight aboard the ISS that has further paved the way for conducting molecular biology experiments on spaceflight (Castro-Wallace et al., 2017). The use of this technology offers a substantial potential for monitoring the microbial population aboard the spaceflight in real time. Several other compact third-generation sequencing technologies can also be adapted to microgravity conditions and deployed in the ISS (Goodwin et al., 2016; McGinn and Gut, 2013). Furthermore, a nucleic acid extraction technology has been recently tested and adapted to operate under microgravity conditions (Urbaniak et al., 2020b). In addition, the ISS harbors several hardware components to conduct microbiology experiments, including growth chambers and instruments to control the exposure of microbes to space radiation (Castro et al., 2013). Therefore, the inclusion of omics technologies in addition to the existing facilities

available on the ISS will allow researchers to conduct experiments followed by sequencing and data analysis in the spaceflight, rather than waiting for sample return to Earth.

A previous report suggested the use of omics, including genomics, proteomics, transcriptomics, metabolomics, epigenomics, exposomics, and metallomics, to establish markers specific to simulated microgravity conditions (Schmidt et al., 2016). To date, the use of genomics, transcriptomics, metabolomics, and proteomics has been of great importance in studying gene regulation at the cellular level, thereby providing insight into altered cellular processes upon exposure to spaceflight or microgravity conditions. For instance, genomic comparison of ISS *Enterobacter bugandensis* isolates with their Earth counterparts revealed the presence of antimicrobial drug resistance genes, including upregulation of efflux pumps (Singh et al., 2018a). These results indicate potential drug resistance properties of these ISS isolates that need to be further confirmed by phenotypic characterization. Another study reported a multi-omics analysis of a model fungus, *A. nidulans*, exposed to space conditions when compared with ground controls. Correlation of the genomics, proteomics, and metabolomics data of spaceflight-exposed *A. nidulans* showed that the introduction of a stop codon in a regulatory gene, *laeA*, resulted in reduced LaeA protein levels, thereby affecting secondary metabolite production (Romsdahl et al., 2019). In another study, proteomic analysis of *A. fumigatus* ISS isolates and its comparison to clinical isolates identified the proteins that are responsible for its adaptation to the space environment. Most of the proteins that were upregulated were involved in stress responses, carbohydrate metabolism, and secondary metabolism (Blachowicz et al., 2019b). However, all these studies relied on the ISS sample return to Earth for analysis. Therefore, several different alternatives that can be adapted to the ISS, for instance, those discussed previously to analyze transcriptome and proteome (Grimm et al., 2014; reviewed in Karouia et al., 2017), should be emphasized for future space research. Furthermore, in addition to genomics, nanopore sequencing has enabled the identification of epigenetic changes in bacteria via detection of DNA base modification at N6-methyladenine (McIntyre et al., 2019). The availability of nanopore sequencer on the ISS can help address epigenetic questions in addition to genetic analysis. Therefore, the inclusion of other omics technologies to study microbial physiology under spaceflight or simulated microgravity conditions can help develop microbial markers under space microgravity conditions. Overall, the correlation of omics with microbial phenotypic responses can help us gain insight into the global changes responsible for the observed phenotype in space conditions. This will further aid in developing precision medicines against pathogenic microbes, similar to that hypothesized for humans in space (Schmidt and Goodwin, 2013), both of which are critical for the safety of future long-term manned space missions.

FUTURE DIRECTIONS

The major goal of the microbiology element in the NASA Science plan involves understanding the effect of spaceflight on microbial life, processes, and dynamics (NASA, 2016). Some of the guiding questions include how the spaceflight influences microbial pathways, microbe-microbe interactions, biofilm formation, virulence, and microbial interactions with other organisms (NASA, 2016). Based on the NASA decadal survey, several microbial tracking experiments have been initiated to understand the microbial persistence on the spacecraft (Checinska Sielaff et al., 2019; Singh et al., 2018b; Venkateswaran et al., 2014). In addition, several experiments have been conducted to understand microbial processes, including biofilm formation (Kim et al., 2013), pathogenicity, and virulence (Crabbe et al., 2013; Ding et al., 2004), as discussed previously. An overview of the key space microbiology experiments is shown in Figure 3. However, the research in spaceflight or simulated microgravity has been limited to only a few microorganisms. This is partly due to the extensive time, resources, and efforts required to conduct experiments on the spaceflight followed by transporting the biological materials back to Earth for further analysis. Therefore, in the future, more efforts should be put toward adapting different technologies, including omics (Bayram et al., 2018; Karouia et al., 2017; Schmidt et al., 2016), to the ISS. Incorporating "omics" in space would provide an answer to many different questions and help understand different aspects of microbial life on the ISS. Further on, to simplify the experiments onboard, emphasis should be given to conducting preliminary experiments on Earth using devices that can simulate spaceflight conditions, which will allow researchers to plan experiments meticulously on the ISS. Another aspect that needs attention is the scarcity of information available on the persistence of viral load and their interaction with other organisms on the spacecraft. Some of the previous studies have reported reactivation of viruses in astronauts, including herpesvirus and Epstein-Barr virus, while onboard the ISS (Mehta et al., 2017; Rooney et al., 2019). Omics can also help obtain information about the persistence of viruses on the ISS so that effective techniques to detect those viruses and countermeasures for the safety of crew health can be developed.
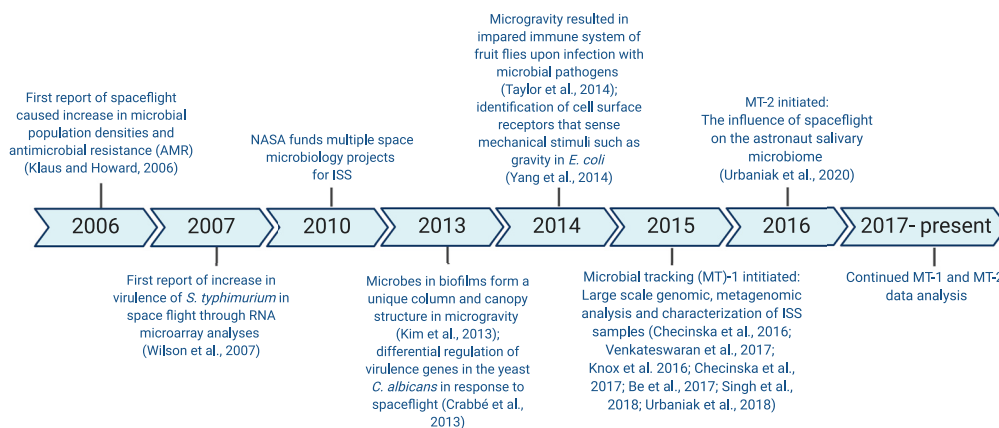
Figure 3. TImeline of key space microbiology experiments

An overview of the space microbiology experiments leading to Microbial Tracking (MT) experiments (MT-1 and MT-2) (Be et al., 2017; Checinska Sielaff et al., 2017; Checinska Sielaff et al., 2016; Crabbe et al., 2013; Kim et al., 2013; Klaus and Howard, 2006; Knox et al., 2016; Singh et al., 2018b; Taylor et al., 2014; Urbaniak et al., 2018, 2020a; Venkateswaran et al., 2017; Wilson et al., 2007; Yang et al., 2013b). The figure has been generated using BioRender (https://biorender.com/).

Despite promising applications of microorganisms in space, information regarding how bacteria and fungi adapt to microgravity and radiation conditions, genetically or physiologically, remains limited. Although increased pathogenicity and virulence are a concern for crew health, microbial activity aboard spacecraft can be beneficial. Therefore, studies moving forward should be aimed at characterizing the advantages of microbial life in space. Life support functions, such as waste degradation, water recovery, and oxygen production, can be carried out by microorganisms, thus, sustaining life in space. In fact, several studies have shown that fungi, bacteria, and cyanobacteria enhance vitamin production, water recycling, air decontamination, and waste management under space conditions (Acevedo-Rocha et al., 2019; Aydogan-Cremaschi et al., 2009; Carillo et al., 2020; Lindeboom et al., 2020; Roberts et al., 2004). In particular, fungal biotechnology can be harnessed such that space stations are self-sustaining with respect to food, pharmaceuticals, waste recycling, and plastic degradation for long-term missions (Cortesão et al., 2020). Challenges ahead may involve developing a balanced microflora that maximizes life support functions and minimizes the threat to crew health in closed space systems.

Another important property of fungi that can be harnessed is their ability to synthesize pigments in the presence of radiations that helps them survive. Fungal species belonging to genera *Cladosporium*, *Paecilomyces*, and *Penicillium* were observed to grow toward the source of radiation at the Chernobyl nuclear accident site, and this property was termed "radiotropism" (Zhdanova et al., 2004). *Cryptococcus neoformans* and *Cladosporium sphaerospermum* exhibited increased growth in the presence of ionizing radiations due to changes in melanin synthesis (Dadachova and Casadevall, 2008). Microorganisms exposed to space conditions encounter similar extreme radiation conditions and therefore develop protective mechanisms to adapt to these conditions. Proteomic characterization of *A. fumigatus* and *A. niger* isolated from the ISS surface showed an increased abundance of Arp1 and AlbA proteins, respectively, that are involved in the synthesis of DHN-melanin (1.8-dihydroxynaphthalene melanin) (Blachowicz et al., 2019b; Romsdahl et al., 2018). Another study as a part of CASIS-NPμG project exposed eight radiation-resistant fungal strains isolated from Chernobyl nuclear accident site to spaceflight condition followed by their genome sequencing and assembly (Singh et al., 2017). Further omics analysis is ongoing to understand the mechanistic basis of the production of bioactive compounds by these fungal species exposed to space conditions, compared with ground controls (unpublished data). This knowledge can help us harness their potential to protect life forms, including protecting humans from harmful radiations on space as well as on Earth.

Although enhanced virulence under space conditions has the potential to negatively impact crew health, it can also be used as a tool to guide the development of novel therapeutics. Drug discovery, modeling of drug pathways, and vaccine development are important areas that necessitate attention for the benefit of human life on space and Earth. Specifically, the discovery of new factors involved in pathogenicity via

microgravity experiments can inform antibiotic drug and vaccine development. For example, the identification of novel virulence genes influenced by microgravity could allow us to more precisely attenuate bacteria using live-attenuated vaccines (Higginson et al., 2016). Traditionally, live-attenuated vaccines reduce virulence by introducing strains with metabolic mutations that slow or inhibit bacterial growth. This approach will help us identify novel therapeutic targets that allow the strain to remain more metabolically fit, thereby resulting in a heightened host immune response. Currently, research is in progress aboard the ISS by National Lab Pathfinder (NLP) missions to develop a vaccine against diarrhea-causing strains of *Salmonella* (Horneck et al., 2010). In addition to virulence genes, the identification of microgravity-sensitive global regulators could guide the development of novel antibiotics. In particular, compounds capable of disrupting these global regulators could inhibit bacteria from sensing their environment and activating virulence factors (Higginson et al., 2016). This approach to disarm bacterial virulence has been successfully utilized against *Citrobacter rodentium* in mice using Regicin, a novel inhibitor of the global regulator RegA (Yang et al., 2013a). Hence, employing such an approach with microgravity-sensitive global regulators could prove beneficial for the design of novel antibiotics. Independent studies have shown that hepatocytes grown in RWVs form three-dimensional colonies characterized by increased and prolonged functionality (Brown et al., 2003; Chang and Hughes-Fulford, 2014). Not only the fact that such 3D culture systems better mimic the microenvironment of the intact liver but also the fact that they can sustain themselves longer than traditional cultures makes them optimal candidates for pre-clinical drug testing (Hammond et al., 2016). These 3D culture systems that mimic human tissues can also be used to study host-pathogen interactions to identify drug targets and, additionally, drug testing. These novel vaccines or drug targets can be used for treatment in spaceflight conditions, and further can be translated for use on Earth to treat an infected individual where the pathogenic organism encounters a similar low-shear environment.

In summary, several research projects are being carried out to utilize the ISS as a test bed for microbiological studies and established MSSF to support microgravity-related scientific experiments. Furthermore, with the advancement of multi-omics, a better understanding of the microbial process is being obtained that will help devise strategies for sustaining healthy human life in future long-term manned missions to Moon, Mars, and Beyond.

AUTHOR CONTRIBUTIONS

S.B., E.S., and N.K.S. wrote and revised the manuscript. K.V. and C.C.C.W. revised and provided feedback on the manuscript. S.B. and C.C.C.W. prepared an outline for the manuscript.

DECLARATION OF INTERESTS

All authors declare no competing interests.

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