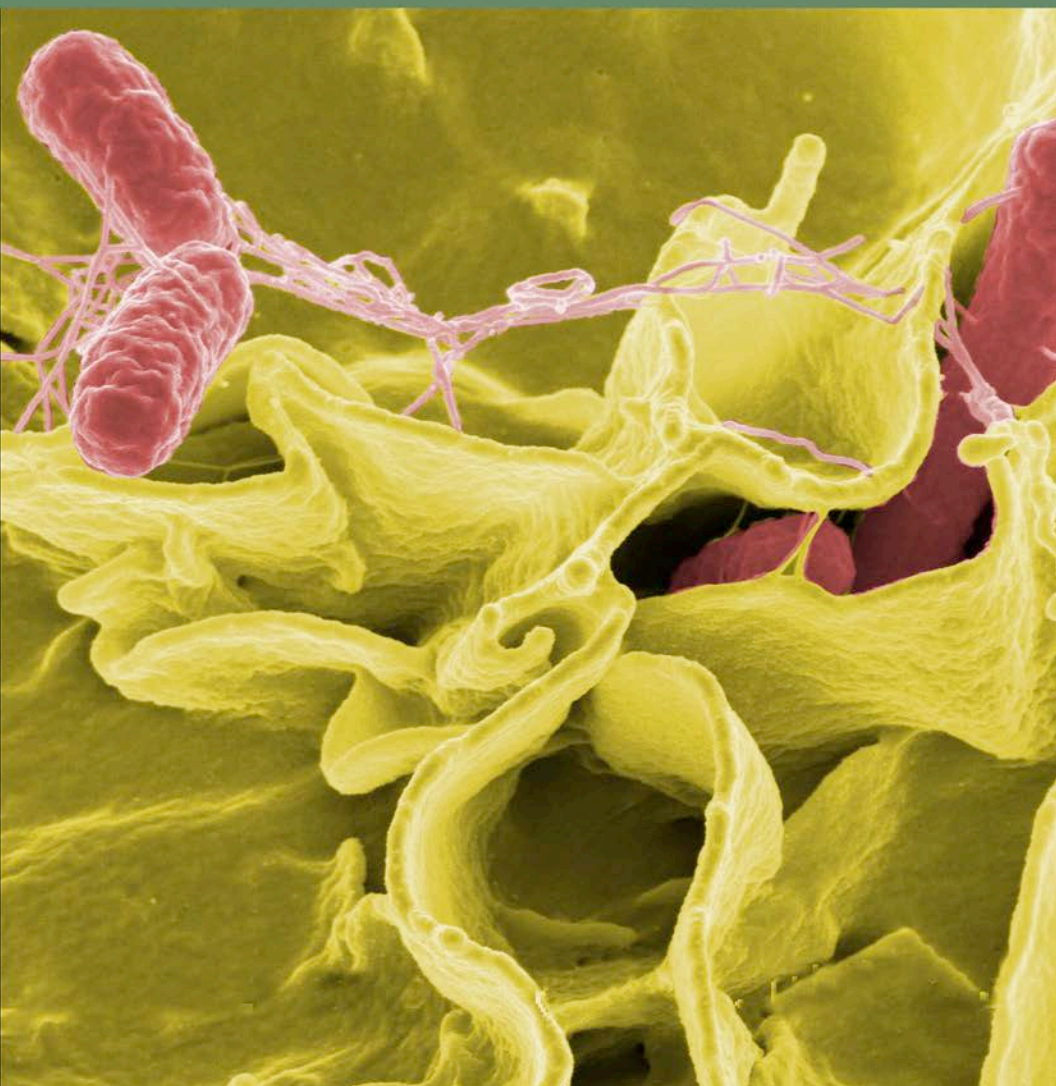
National Aeronautics and Space Administration

A Researcher's Guide to:

INTERNATIONAL SPACE STATION

Microbial Research



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Cover and back cover:

a. Color-enhanced scanning electron micrograph showing Salmonella typhimurium (red) invading cultured human cells Credit: Rocky Mountain Laboratories, NIAID, NIH Source: [http://www2.niaid.nih.gov/biodefense/public/images.htm NIAID]

b. Back Cover 1: Computer-generated bacteria image.

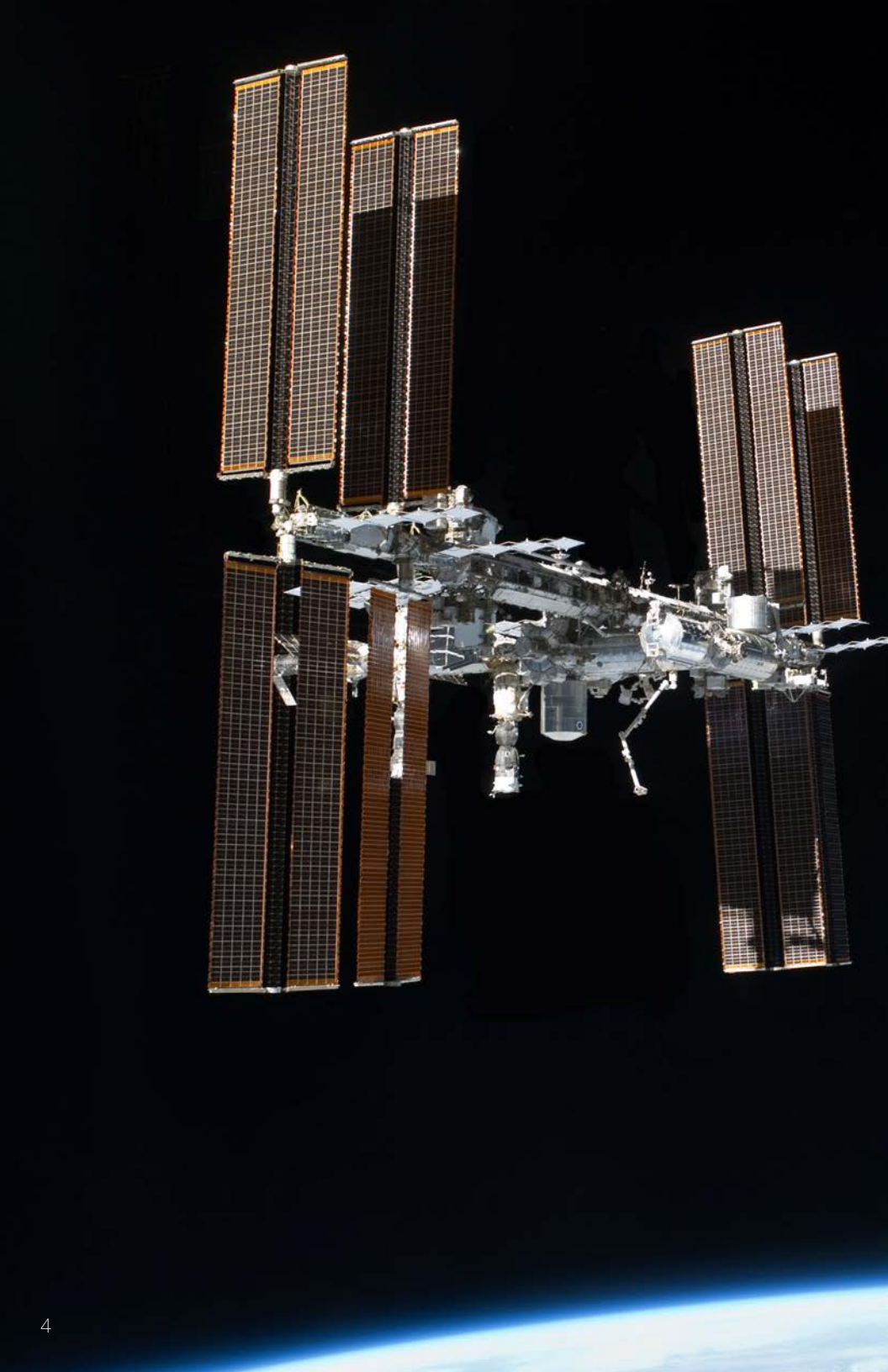
c. Back Cover 2: Computer-generated virus image.

The Lab is Open

Orbiting the Earth at almost 5 miles per second, a structure exists that is nearly the size of a football field and weighs almost a million pounds. The International Space Station (ISS) is a testament to international cooperation and significant achievements in engineering. Beyond all of this, the ISS is a truly unique research platform. The possibilities of what can be discovered by conducting research on the ISS are endless and have the potential to contribute to the greater good of life on Earth and inspire generations of researchers to come.

As we increase utilization of ISS as a National Laboratory, now is the time for investigators to propose new research and to make discoveries unveiling novel responses that could not be defined using traditional approaches on Earth.

An astronaut holding a Microbial Air Sampler (MAS) Petri Dish on the ISS. The MAS is used for the collection of cabin air atmosphere for evaluation of the microbial load.



Unique Features of the ISS Research Environment

1. **Microgravity**, or weightlessness, alters many observable phenomena within the physical and life sciences. Systems and processes affected by microgravity include surface wetting and interfacial tension, multiphase flow and heat transfer, multiphase system dynamics, solidification, and fire phenomena and combustion. Microgravity induces a vast array of changes in organisms ranging from bacteria to humans, including global alterations in gene expression and 3-D aggregation of cells into tissue-like architecture.

2. **Extreme conditions** in the ISS environment include exposure to extreme heat and cold cycling, ultra-vacuum, atomic oxygen, and high energy radiation. Testing and qualification of materials exposed to these extreme conditions have provided data to enable the manufacturing of long-life reliable components used on Earth as well as in the world's most sophisticated satellite and spacecraft components.

3. **Low Earth orbit** at 51degrees inclination and at a 90-minute orbit affords ISS a unique vantage point with an altitude of approximately 240 miles (400 kilometers) and an orbital path over 90 percent of the Earth's population.
   This can provide improved spatial resolution and variable lighting conditions compared to the sun-synchronous orbits of typical Earth remote-sensing satellites.

Table of Contents

Microbiology Research Priorities on the ISS

We are on the cusp of the next giant leap in space exploration and related scientific research. The private sector has reinvigorated the space race, and several countries have affirmed their intentions of developing a robust human spaceflight program. The United States has targeted full utilization of the International Space Station (ISS) and set its exploration sights beyond low-Earth orbit. As we determine our destinations for the next generation of spaceflight, several questions remain to be answered as to the effects of the spaceflight environment on human physiology and the microorganisms that will, without question, accompany them. The answers to these questions have the potential to benefit not only those who travel in space, but also provide knowledge to benefit those who remain on Earth.

A human is both an individual organism and an entire ecosystem, including microorganisms in, on, and around them in which the human cells are greatly outnumbered by the microbial cells. The microbial inhabitants in and on the person outnumber the human cells 10 to 1. For the most part, these microorganisms are beneficial to their human host or otherwise innocuous. Given the right opportunity, either a shift in the environment of the host or the invasion to a new location within the host, can cause the microorganisms to become pathogenic. Therefore, potential pathogens have been present on all NASA missions (Rogers 1986, Castro et al. 2004). Protective measures such as stringent microbial monitoring, the use of freeze dried foods, and preflight crew quarantine have been used to decrease the risk of infectious disease during a mission (Johnston 1969, Rogers 1986). Over the past 50 years, a combination of operational experience, spaceflight and ground-based research have provided tremendous insight into infectious disease risk as well as necessary preventative measures (Johnston 1969, Taylor 1972, Taylor 1976, Facius 1978, Fang et al. 1997, Nickerson et al. 2004, Ott 2004). Significant strides have been made to define and mitigate the source of microbial contamination aboard spacecraft and to document the responses of numerous microorganisms to the spaceflight environment. This collection of experience and research data also helped in the identification of critical gaps in our understanding of how this environment impacts microbial ecology, the microbial genotypic and phenotypic characteristics, and their interactions with plant and animal hosts. As we look toward human interplanetary exploration, the importance of this knowledge has been recognized. With the increases in both the occupancy and duration of humans aboard the ISS, these knowledge gaps are becoming better defined. With the laboratory platform aboard ISS, many of these gaps for future spaceflight can be understood.

There is much to be gained by employing the microgravity environment of spaceflight as a basic research platform. Life on Earth evolved in the presence of gravity. Therefore, performing research in the reduced gravity of spaceflight holds the potential to determine how this physical force shaped terrestrial life. Previous spaceflight and ground-based spaceflight analog research has established that even microorganisms, the smallest Earth-based life forms, are intrinsically able to respond to changes in this force (Dickson 1991, Mishra 1992, Nickerson et al. 2000, Nickerson et al. 2004). While over 50 years of microbial research has been performed in spaceflight, a thorough understanding of microbial responses to spaceflight culture and how the spaceflight environment stimulates these responses is only beginning to be understood. Microgravity as a research tool, coupled with current molecular technology, provides researchers the opportunity to establish how variations in this physical force affect microbial life at the cellular, molecular and evolutionary levels. This potential is not surprising as innovative answers to complicated medical, environmental and agricultural questions have arose from assessing the properties of microorganisms in many extreme environments on Earth (Nickerson et al. 2004). Similarly, the study of microbes in the spaceflight environment holds considerable potential for future, basic research and industrial applications. Investigations into microbial ecology, genotypic and phenotypic properties, and the infectious disease-causing potential of microorganisms in the spaceflight environment, may unveil novel mechanisms that could not be elucidated using traditional approaches on Earth, where gravity may be restricting our discovery of unique cellular responses.

Because of both the gaps in our knowledge as to how the spaceflight environment affects microorganisms, and the immense prospects associated with conducting research in this environment, the National Research Council (NRC) Committee for the Decadal Survey on Biological and Physical Sciences in Space 2011 report "Recapturing a Future for Space Exploration: Life and Physical Sciences Research for a New Era," recommended, with emphasis, on establishing a coordinated, large-scale microbial observatory program within the ISS platform. Specifically, the committee prioritized:

1. The establishment of a microbial observatory program on the ISS to conduct long-term, multigenerational studies of microbial population dynamics.

2. The establishment of a robust spaceflight program to research analyzing plant and microbial growth in spaceflight environments and physiological responses to the multiple stimuli encountered in those environments.

3. The development of a research program aimed at demonstrating the roles of microbial-plant systems in long-term life support systems.

The ISS as a Microbial Observatory

The original concept of microbial observatories as stated by the United States National Science Foundation (NSF) was to study and understand microbial diversity over time and across environmental gradients. A key element of diversity studies is to seek out information about previously unidentified microbes in the various environments in which an observatory is established. The ISS is an excellent experimental system for studying changes in diversity over time under controlled conditions. While the discovery of previously unidentified microorganisms is unlikely, the ISS is an ideal setting to study microorganisms in a complex contained, isolated, "island-like" ecosystem. Many scientific studies have focused on either complex ecosystems that are not well-controlled in a classical experimental sense or very simple ecosystems that are well-controlled but severely limited in dimension and/or diversity. To date, complex controlled ecosystems have not persisted for long periods of time; so studying microbial dynamics within them has been necessarily a short-term endeavor. Since its initial launch in the commencement of construction in orbit, ISS has been a relatively closed system with the only inputs of microorganisms to the ISS arriving with the occasional resupply from Earth along with crew changes during ISS increments. Factors influencing microbial growth and response are well monitored and recorded, including environmental conditions such as temperature and humidity, as well as crew diet and activity. New ISS modules and transported cargo are also evaluated for microbial diversity and concentration.

These punctuated, highly monitored introductions of additional microbes provide a platform from which the human/environmental microbiome can be uniquely investigated. The ISS offers opportunities to study the dynamics of microbial populations and communities in the absence of mass uncontrolled immigration of uncharacterized organisms from unknown sources. While other systems may have some of the characteristics of ISS, none can match this platform's unique isolation from contaminating contacts. While the ISS is not a completely closed system, the low frequency of exchange of materials with the outside and the potential for characterization of the microbiology of materials brought to station makes control of microbial inputs to this unique system much more achievable. This isolation provides the opportunity to gain insight into the interactions between humans and environmental organisms and changes in microbial communities through mutation or genetic exchange with minimal external interference.

Research Scope of an ISS Microbial Observatory

NASA Space Life Sciences proposes to support research that will discover and characterize fundamental mechanisms used by microorganisms and microbial communities to adapt to the diverse challenges of the spaceflight environment. That research will use advanced molecular biology, genomics, bioinformatics, and cultivation technologies to understand spaceflight microbial community fundamental properties, interactions with humans, and adaptation to other planets or interplanetary space.

Table 1. Opportunities for Microbial Research on the ISS

Subject Area	Potential Research Questions for ISS Investigations
Microbial Physiology	Does the spaceflight environment cause alterations in microbial growth profiles; response to stressors; motility; and microbial metabolism?
Microbial Ecology	Does the spaceflight environment cause alterations in the relative roles of microbial ecology and evolution; the human and plant microbiome as a subset of the ISS microbiome; microbial interactions with the environment over time; microbial population dynamics; dynamics of succession; stabilities of closed-model communities (not ambient); mechanisms of community change/biogeography, selection pressure, generational aspects within selected microbes versus communities; microbial populations occurring naturally in the environment (air, surfaces, water); and the spread of identified strains as a result of the spaceflight environment?
Molecular Microbiology	Does the spaceflight environment cause alterations in microbial genomic diversity; genomic evolution; microbial sensing; and the microbial transcriptome, proteome, or metabolome?
Microbial Interactions	Does the spaceflight environment cause alterations in microbe-microbe interactions; host-microbe interactions; plant-microbe interactions; and biofilm formation or function (single species and mixed populations)?

Benefits of an ISS Microbial Observatory

There are numerous benefits from enabling and expanding microbial diversity research on the ISS. From a microbial ecology perspective, research in this area has the potential to play a major role in the development of the microbial ecology of indoor environments (Corsi et al. 2012). Millions of dollars have been invested to understand the microbial ecology of terrestrial and marine environments, yet we know very little about the microbial ecology of the environment we are most

intimate with—buildings. Humans in the developed world spend more than 90 percent of their lives indoors (Klepeis et al. 2001) where they are exposed to airborne and surface microorganisms. These microbial communities might be intimately connected to human health (Burge 1995, Mitchel et al. 2007, Srikanth et al. 2008). Examples include the spread of acute respiratory disease (Cohen et al. 2000, Smith 2000, WHO 2007, Glassroth 2008) and the increase in the occurrence of asthma symptoms (Ross et al. 2000, Eggleston 2009, Schwartz 2009). Historically, buildings were designed to keep microbes outside using barriers and elaborate, energy-intensive mechanical systems. Yet we don't fully understand the causes and consequences of microbial diversity in indoor environments. Indoor ecology research challenges scientific paradigms for at least two reasons. First, it reframes the modern field of microbial ecology to include manmade indoor environments. Second, it challenges the perspective that the indoor environment is a place for chemistry, physics, and infection control research. Buildings are ideal for ecology research because they are accessible, "island-like," and they can be experimentally manipulated.

The ISS offers an unprecedented opportunity to advance indoor ecology research. It provides an experimental platform for controlling two of the largest contributors to indoor microbial diversity: ventilation source and occupancy load. Research has shown that ventilation source significantly impacts microbial diversity indoors, with mechanically ventilated rooms harboring more potential airborne pathogens than naturally ventilated rooms (Kembel et al. 2012). It has also been demonstrated that human occupancy load impacts the abundance and diversity of airborne microbes (Qian et al. 2012). Together, these findings suggest that tremendous knowledge would be gained by conducting experiments in a highly controlled environment like the ISS where the ventilation source and occupancy load can be systematically analyzed. By sampling the built environment microbiome and human microbiome in the ISS jointly, it would be possible to tease apart how microbes are exchanged among humans, indoor air, and indoor surfaces.

Another unique benefit of the ISS as an experimental, complex closed ecosystem is that over time the microbial communities present on the space station are likely to become more and more dominated by human-associated microbes. Within the confines of the ISS, the environmental control and life support system maintains a homeostatic environment suitable for sustaining the human crew for six-month increments. This environment also acts to sustain and select a human-associated

microbiome that persists on the ISS across all the increments. As has been observed in other environmentally controlled and human-engineered constructs like office buildings and airplanes, the microbiome will change in diversity (i.e., number of different types or species of microorganisms in the spacecraft) and structure (i.e., the relative composition of different types or species) over time. The relative abundance of human-associated bacteria, including those that could potentially cause disease, is higher indoors than outdoors. As the ISS is relatively closed, the microbial diversity is relatively stable throughout the interior of the station, such that the dispersion of new microorganisms can be tracked and impact of their addition on the "station" community can be evaluated. This premise may also be possible for investigations into changes in the astronaut microbiome

Finally, an ISS Microbial Observatory would provide an opportunity to broaden our understanding of the unique microbial responses of microorganisms cultured during spaceflight. ***This aspect of the ISS Microbial Observatory distinguishes it from any other available facility, as no other platform can provide this microgravity environment.*** As the microorganisms are adapting their responses to this novel environment, information can be gathered that provides unique insight into microbial regulation and function that cannot be discerned using traditional methodology on Earth.

The use of the ISS as a microbial observatory would drive experiments that could decrease infectious disease risk during the human exploration of space, advance the application of beneficial purposes for microorganisms (e.g., waste remediation, probiotics), and provide unique insight into basic microbial functions and interactions that could be translated to studies for scientists and commercial entities on Earth. Translation of spaceflight findings has already begun to take place as scientists and corporations are investigating the use of ISS microbial findings to better understand virulence profiles, antibiotic and disinfectant resistance, biofilm formation, and biodegradation properties.

As NASA travels beyond low-Earth orbit to planets such as Mars, insight from an ISS Microbial Observatory will impact our approach to exploration. Understanding how spaceflight and gravity alter microbial responses, their exchange of genetic material, and their expected concentrations and distribution will be vital in our search for extraterrestrial life and concurrent planetary protection.

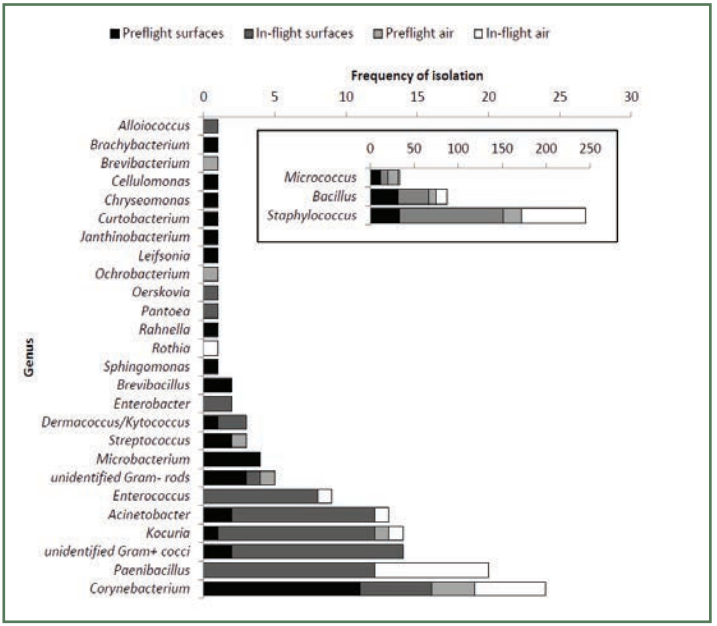
Microbial Diversity of Spaceflight Crew and Craft

With the exception of sending microorganisms into orbit for research purposes, significant care is taken to reduce the levels of microbes in the spaceflight environment. Stringent preflight microbiological monitoring and remediation of NASA spacecraft has been performed throughout the human spaceflight program (Johnston 1969, Rogers 1986, Castro et al. 2004). However, the microbiota of the crew members, in combination with an inability to ensure complete sterility of the craft and cargo, results in the coexistence of humans and microorganisms in the spaceflight environment. In-flight data acquired during Apollo, Skylab, and the Mir Space Station missions increased our knowledge of the impact of spacecraft habitation on the crew and vehicle microbiota. The findings from these early spaceflight programs were critical to the design of latter spacecraft and in establishing microbiological acceptability limits for the in-flight environment. While this information, in combination with insight gained from the space shuttle and ISS programs, has proven critical in our approach to mitigating microbial risk to crew members and their vehicle, great numbers of questions remain.

Microbiological evaluations of the crew members have been in place since the first manned Apollo flight with the goal of characterizing the microbial load of astronauts preparing for lunar surface exploration (Taylor 1972). During early Apollo missions, a thorough microbial baseline was established for each astronaut to facilitate the identification of any possible terrestrial contaminants in returned lunar samples (1969). Studies conducted during the later Apollo missions were designed to identify and prepare for possible microbial-associated issues arising as a result of the more lengthy Skylab program (Taylor 1972). The findings of these early investigations included identifying trends such as increases in the number of sites on a crew member's body that organisms were isolated from and the quantity of those organisms as well as increased levels of microbes in the environment (1969, Johnston 1969, Taylor 1972). These early studies also documented the incidence of microbial transfer between crew members and the spacecraft environment (Taylor 1972). The knowledge gained resulted in operational and engineering activities to control the environment of the crew concerning crew contacts (quarantine), food, water, and air.

Most of our understanding of the microbial diversity aboard spacecraft has relied on the culture of microorganisms using a relatively few types of growth media. Generally, the environmental data indicate that the potable water, air, and surfaces to which the crew is exposed are free of obligate pathogens; however, opportunistic pathogens such as *Pseudomonas aeruginosa, Stenotrophomonas maltophilia,* and

Staphylococcus aureus are not uncommon (Pierson et al. 1996, Castro et al. 2004, Pierson 2012). Spaceflight food is another source of microorganisms aboard spacecraft. While the incidence of contamination is low, preflight analyses of food samples have indicated the presence of organisms such as *Salmonella enterica* serovar Typhimurium (S. Typhimurium), *Staphylococcus aureus, Enterobacter cloacae* and *Cronobacter sakazakii* (unpublished data). Figure 1 details the relative abundance of bacterial and fungal strains isolated from the air and surfaces of the ISS before and during flight. As these findings are based on cultured organisms, only a part of the picture of the microbial diversity of spacecraft has been captured. By coupling current molecular methods with the ISS platform, a higher resolution of this picture has the potential to be viewed.



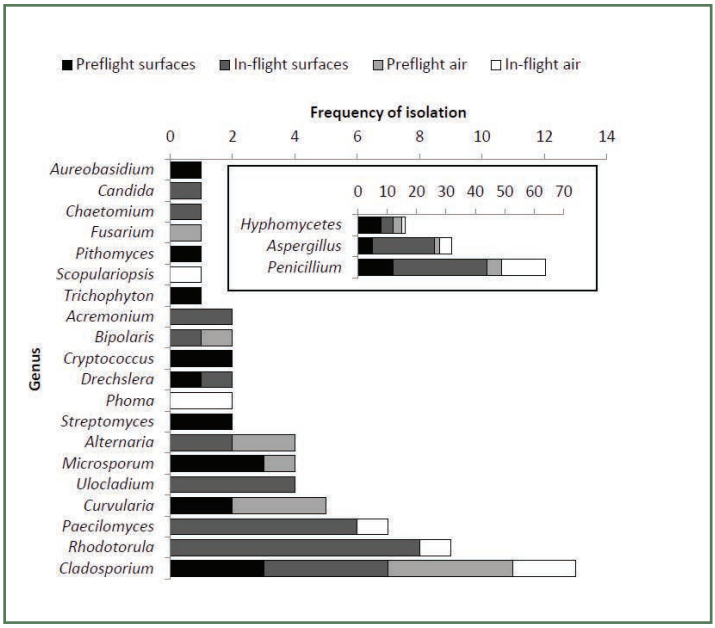


Figure 1. The abundance of A) bacterial and B) fungal strains isolated from air and surfaces from the International Space Station environment before and during flight. Isolates are categorized by genera and relative abundance (Pierson 2012).

While multiple preflight measures are in place to limit microbial contamination aboard spacecraft, the limitations of these conventional monitoring methods were demonstrated by a comprehensive media-based and microscopic analysis of microorganisms isolated from free-floating water collected behind panels aboard the Mir Space Station (Ott 2004). Several medically significant organisms that were not commonly isolated during standard operational monitoring were identified including *Legionella* species, *Serratia marcescens*, and *Escherichia coli*. Further microscopic examination of these samples revealed the presence of amoeba resembling *Acanthamoeba* or *Hartmanella* species and ciliated protozoa resembling *Stylonychia* species (Ott 2004). This finding reinforced the need for a more thorough investigation of the microbial diversity of spaceflight habitats, especially at time points later in their service life.

Preventive measures during spaceflight missions continue to provide protection to the crew. While the duration of the preflight quarantine period, which reached its peak during the Apollo program, has been significantly reduced for crew members (currently, approximately 10 days), it still exists with the goal of limiting the number of obligate pathogens that are carried into the spacecraft by the crew. Spaceflight food is routinely screened for the presence of harmful microorganisms; the ISS is equipped with HEPA filters, and the water system is treated with biocides all to reduce the infectious disease risk to the crew. The risk of obligate and opportunistic pathogen carriage has not been eliminated. Our understanding of the crew microbial flora is primarily based on traditional culture-based methodology, which provided adequate health care; however, a thorough understanding of alterations in the crew microbiome using current genetic identification, transcriptomic analysis, or other advanced technologies has not been accomplished. While a number of studies have been initiated, the alterations in the human microbiome are tremendously understudied and hold the potential to greatly advance our knowledge of crew health during a spaceflight mission.

Microbial Responses to Spaceflight

The short generation time of microorganisms makes them uniquely suited for studies assessing responses to altered environmental conditions. Microbial cells were among the first Earth-based life forms to be sent into the microgravity environment of space. These early investigations established that bacteria and fungi remained viable and capable of reproducing while also setting a precedent for conducting research in the spaceflight microgravity environment. Although more than 100 spaceflight experiments involving microorganisms have been conducted over the past 50 years, significant gaps in our knowledge as to how this environment impacts microbial ecology, microbial genotypic and phenotypic characteristics, and host-microbe interactions remain.

A Brief History of Microbial-based Spaceflight Research

In 1960, prior to the flight of Yuri Gagarin, scientists from the Union of Soviet Socialist Republics (USSR) launched *E. coli, Aerobacter aerogenes*, and *Staphylococcus* into orbit aboard an unmanned satellite (Zhukov-Verezhnikov 1962, Zhukov-Verezhnikov 1963). It was this experiment that lead to the conclusion that the microgravity environment of space did not affect the viability of the microorganisms (Zhukov-Verezhnikov 1962, Zhukov-Verezhnikov 1963). In an important subsequent experiment, the USSR launched *E. coli* aboard Vostok 2 in 1961, which resulted in the identification of a variant colony type that was reported to be a result of spaceflight factors (Klemparskaya 1964). In 1967, NASA launched the unmanned Biosatellite 2, which exposed various biological specimens including *E. coli* and *S.* Typhimurium to the microgravity environment of space for 45 hours (Mattoni 1968, Mattoni 1971). For both microorganisms, an increase in population density was noted for the in-flight samples (Mattoni 1968, Mattoni 1971). *Bacillus subtilis* was cultured aboard Apollo 16 and 17 and resulted in the finding that microgravity did not affect the developmental process of spore formation (Bucker 1975). However, when assessed after culture aboard the Apollo-Soyuz Test Project, the colony forming ability of *B. subtilis* spores was found to be reduced among spaceflight samples (Facius 1978). With evidence mounting that bacteria were able to sense and respond to the microgravity environment of spaceflight, the concern of both the U.S. and USSR space programs shifted to how these variations could impact crew health. Over the course of numerous spaceflights, researchers from various countries analyzed changes in antibiotic resistance in *E. coli* and *S. aureus* (Tixador 1983, Tixador et al. 1985, Tixador et al. 1985, Lapchine 1987). The minimal inhibitory concentration (MIC) of oxacillin, chloramphenicol, and erythromycin for *S. aureus* and colistin and kanamycin for *E. coli* were evaluated among in-flight cultures as

compared to controls (Tixador et al. 1985, Tixador et al. 1985). These investigations documented increased bacterial resistance to all antibiotics tested for both *S. aureus* and *E. coli*. The researchers observed a thickening of the cell wall that accompanied the increase in resistance of *S. aureus* once returned from flight (Tixador et al. 1985, Tixador et al. 1985). Various other microbial properties were recorded during this time including increased conjugation in *E. coli* (Ciferi 1988) and increased growth kinetics in *B. subtilis* (*Mennigmann and Lange 1986*) in response to microgravity.

The space shuttle era brought with it an enhanced capability to perform biological research within the microgravity environment of space and to delve further into the implications to human health. In 2006, taking advantage of this opportunity, investigators launched several microorganisms including *S.* Typhimurium aboard *Space Shuttle Atlantis* (STS-115) in an attempt to define the impact of spaceflight culture on the disease-causing potential of the microorganisms (Wilson et al. 2007, Crabbe et al. 2011). The results of the study were dramatic, with the researchers reporting that mice infected with bacteria grown in-flight displayed a decreased time to death, increased percent mortality, and decrease in the lethal dose (Figure 2 D) (Wilson et al. 2007). Analysis of the fixed returned samples revealed differential expression of a large number of genes and identified a regulatory protein that was mechanistically associated with the spaceflight response of the organism (Wilson et al. 2007). This was the first report elucidating both the molecular response connected with a regulatory mechanism and alterations in bacterial virulence as a consequence of growth in the spaceflight microgravity environment.

To confirm these findings and further our understanding of factors influencing spaceflight culture-mediated changes in virulence, a follow-up investigation was performed on *Space Shuttle Endeavor* (STS-123) to again assess the response of *S.* Typhimurium to the spaceflight environment. This set of experiments included culturing *S.* Typhimurium in various different types of growth media in the spaceflight environment (Figure 2 E). The findings from this spaceflight experiment confirmed the previous reports of increased virulence of the bacteria (Wilson et al. 2008). Furthermore, the data from this assessment revealed that media ion concentration dramatically influences the spaceflight-related virulence response of *S.* Typhimurium (Wilson et al. 2008).

In addition to microgravity, the spaceflight environment has a unique radiation background. Several spaceflight experiments have investigated the impact of this radiation on microbial organisms (de Serres 1969, Berry and Volz 1979, Bouloc and D'Ari 1991, Horneck et al. 2010); however, our understanding of topics such as

alterations in mutational rates and how these mutations could alter the phenotype of the organisms is generally understudied.

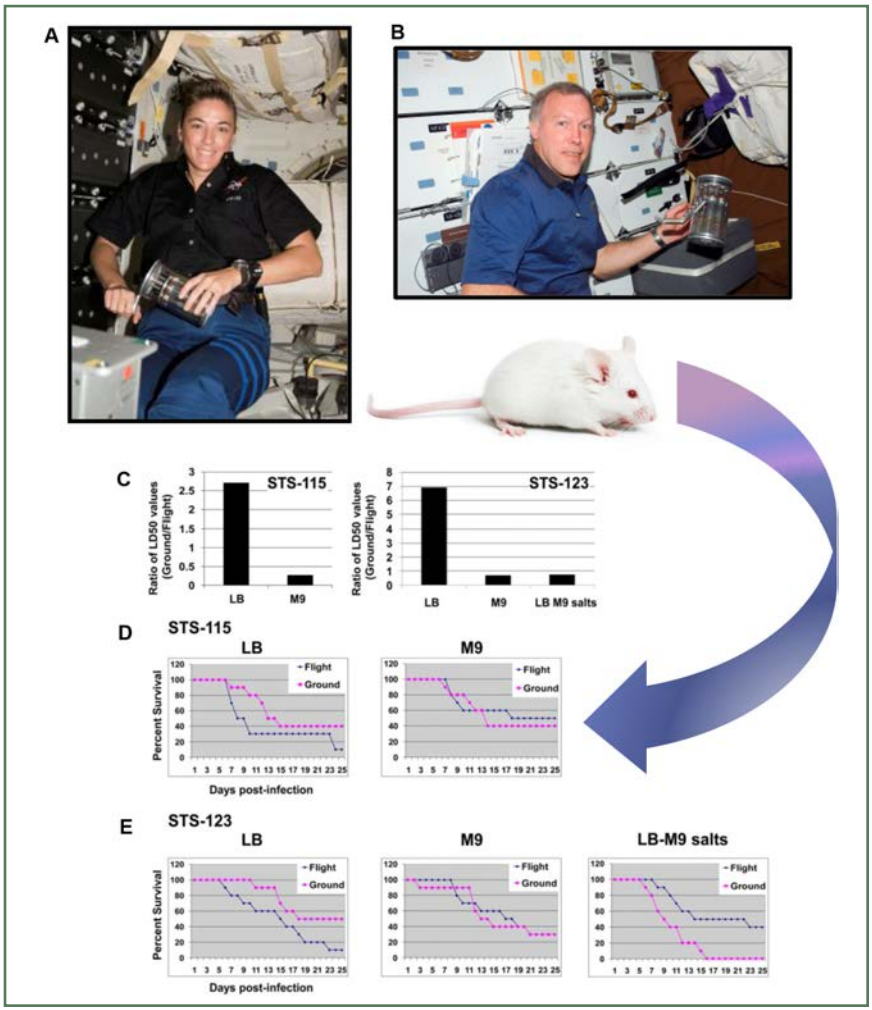


Figure 2. Astronauts A) Heidi Stefanyshyn-Piper activates S. *Typhimurium to grow in-flight during the MICROBE experiment on STS-115 and B) Don Gories activates* S. *Typhimurium to grow in-flight as part of the MDRV experiment on STS-123. After growth in the spaceflight environment, the bacterial samples were returned to Earth and immediately used in a mouse model of salmonellosis. The results from both experiments revealed that* S. *Typhimurium becomes more virulent as a result of growing in the microgravity conditions of space. The experiments also described the composition of the growth medium as an important factor controlling the change in virulence (Wilson et al. 2007, Wilson et al. 2008).*

Initiating Ground-Based Research – Spaceflight Analogs

Microgravity cannot be created on Earth; however, aspects of the microgravity environment can be mimicked by use of ground-based simulators. Numerous ground-based methods of simulating the microgravity environment of spaceflight have been developed and implemented to overcome the constraints that accompany biological gravitational research. Ground-based simulators have proven indispensable as tools for preparing spaceflight experiments and have generated independent investigations. Parabolic flights and drop towers are means of providing "free fall" for a limited amount of time; to enable analysis of microbial response to aspects of the microgravity environment for greater amounts of time, other analogs have been developed using a variety of technology such as clinostats, rotating-wall vessels, random positioning machines, and magnetic levitation (Klaus 2001, Nickerson et al. 2004, Herranz et al. 2013). While these simulators do not eliminate the force of gravity, they reproduce many characteristics of the environment produced in true microgravity.

An Example of a Spaceflight Analog – The Rotating Wall-Vessel Bioreactor

Each of the spaceflight analog systems has both unique advantages and disadvantages (Klaus 2001, Buels et al. 2009, Dijkstra et al. 2011). Of the simpler systems, the rotating-wall vessel (RWV) bioreactor has been increasingly used to enhance our understanding of microbial responses that may be occurring during spaceflight (Fang et al. 1997, Nickerson et al. 2000, Lynch et al. 2004, Nauman et al. 2007, Crabbe et al. 2008, Castro et al. 2011). The RWV (Figure 3 A) is an optimized form of suspension culture in which cells are grown in physiologically relevant, low-fluid-shear conditions. A cell in liquid media in microgravity experiences two unique aspects important in modeling this environment: 1) remaining in a constant state of suspension and 2) experiencing a quiescent surrounding, devoid of shearing, turbulent forces (Klaus et al. 1997). It is these aspects of the microgravity culture environment that the RWV bioreactor effectively models.

The components of the RWV bioreactor system include the vessel, rotation base unit with oxygen pump, and power supply. The vessel is a thin, cylindrical disc to which the cell culture media is introduced by syringe via ports on the vessel's face. Once attached to the base unit, the power supply is turned on initiating rotation

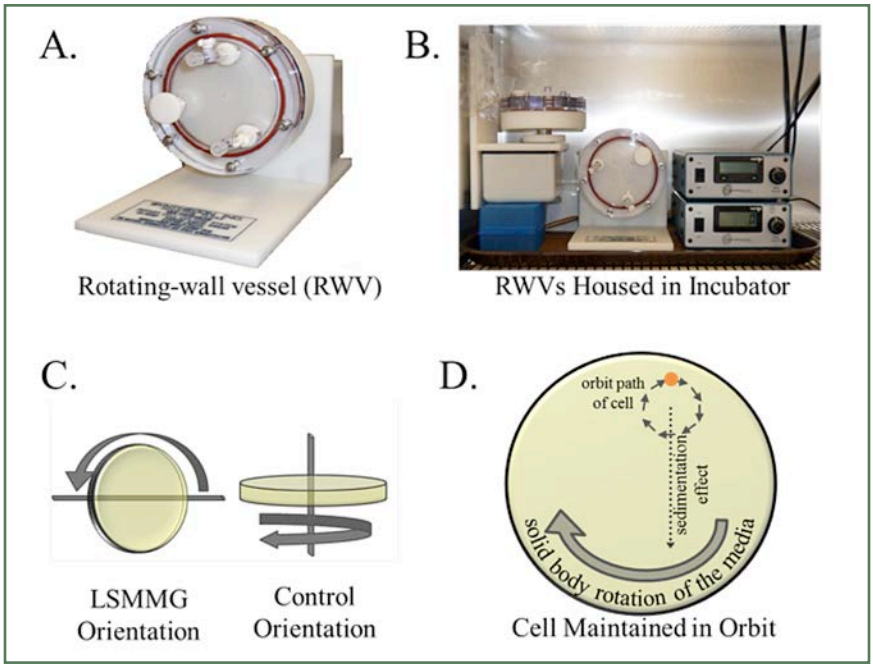


Figure 3. The Rotating-Wall Vessel Bioreactor (Synthecon, Houston, Texas). (A) Image of the NASA-designed RWV apparatus. (B) RWV culture system in the incubator with their respective base units and power supply systems. (C) The altered positioning of the RWV that results in the two culture orientations, depicting the axis of rotation. The LSMMG environment is achieved by rotation of the RWV on an axis parallel to the ground, whereas the axis of rotation in the control orientation is perpendicular to the ground. (D) Depiction of the orbital path of a cell when cultured in the LSMMG orientation. The combination of the sedimentation effect, whereby gravity and lack of motility causes a cell to settle to the bottom of the vessel, and the clock-wise solid body rotation of the media results in the continuous suspension of the cell in an orbit.

of the vessel a supply of oxygen through a gas permeable membrane on the inner backside of the vessel. The entire system can be housed in an incubator to allow for optimal cell growth at a fixed temperature (Figure 3 B). As the fully filled vessel rotates, its rotational velocity is transferred radially inward until the relative fluid motion ceases at which point, solid body rotation of the fluid is achieved (Klaus 2001). A cell within this environment experiences the sedimentation effect imparted by gravity. As it begins to fall toward the bottom of the vessel, "settle out," it is carried back upward by the solid body rotation of the media and, thus, remains suspended in the fluid in an orbital path (Figure 3 D), thereby modeling the first aspect of the microgravity environment described above.

During culture in the RWV bioreactor, a microorganism experiences a quiescent, low-shear, low-turbulent environment analogous to the second aspect of spaceflight. As it is important to note the low-shear effects of the fluid on the cells, the term Low-Shear Modeled Microgravity (LSMMG) has been adopted for use in accurately

describing the environment produced by the RWV bioreactor (Wilson et al. 2002).

In order to assess the response of microorganisms to the LSMMG environment, a suitable control culture is required. A standard static or shake flask culture of the bacterium in question would not suffice as multiple variables, such as aeration, would be altered. Therefore, early analysis with the RWV vessels demonstrated that an optimal control could be achieved by simply altering the orientation of the vessel to rotate on an axis perpendicular to the ground (Figure 3 C) (Fang et al. 1997). Because of the altered orientation of the RWV, the bacterial cell is no longer suspended in the fluid and the low-shear condition has been disrupted (Fang et al. 1997). The use of a reoriented vessel serving as the control has previously been utilized and validated by multiple investigators (Fang et al. 1997, Nickerson et al. 2000, Nickerson et al. 2003, Crabbe et al. 2010, Castro et al. 2011).

Insights Gained from Microbial Culture Within the Rotating Wall-Vessel Bioreactor

The RWV bioreactors were initially intended as a spaceflight analog for eukaryotic cells (Wolf 1991, Hammond and Hammond 2001) but have since been used to examine bacteria (Fang et al. 1997, Nickerson et al. 2000, Crabbe et al. 2008, Castro et al. 2011), fungi (Johanson et al. 2002), and archaea (Dornmayr-Pfaffenhuemer et al. 2011) in response to this environment. In the mid-1990s, Fang and colleagues were the first to put a bacterium inside the RWV and were primarily focused on the effects of LSMMG on secondary metabolite production (Fang et al. 1997, Fang et al. 1997). Over the course of their studies, they noted that the modeled microgravity environment of the RWV did not alter gramicidin production from *Bacillus brevis* (Fang et al. 1997), decreased beta-lactam production by *Streptomyces clavuligerus* (Fang et al. 1997), inhibited *Streptomyces hygroscopicus'* production of rapamycin (Fang et al. 2000), and prevented microcin B17 production from *E. coli* (Fang et al. 2000). A summary of certain bacterial, fungal and archaeal responses to the simulated microgravity conditions within the RWV bioreactors since the work of Fang and colleagues can be found in Table 2.

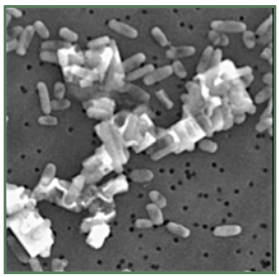
Pioneering work by Nickerson and colleagues expanded this area of research by connecting the LSMMG response of an enteric pathogen, *S.* Typhimurium, to a human host and the spaceflight environment (Nickerson et al. 2000, Wilson et al. 2002, Wilson et al. 2002). The conditions within the RWV were found to have profound effects on the behavior of *S.* Typhimurium including an increase in its virulence potential (Nickerson et al. 2000). Mice challenged with

Table 2. Microbial Responses to Modeled Microgravity

Microorganism	Response to Modeled Microgravity within the RWV Bioreactor	Reference
S. Typhimurium χ^{3339}	- Increased: virulence in a mouse model; resistance to acid, thermal, and osmotic stress; macrophage survival - Decreased: LPS production; resistance to oxidative stress; Hfq expression - Differential gene expression	Nickerson, 2000 Wilson, 2002 Wilson, 2002b Wilson, 2007
S. Typhimurium *14028*	- Increased: virulence in a mouse model and cellular invasion - Differential gene expression	Chopra, 2006
E. coli AMS6	- Increased biofilm formation and resistance to osmotic, ethanol and antibiotic stress	Lynch, 2006
E. coli E2348/69	- Increased intimin production	Carvalho, 2005
E. coli MG1655	- Decreased growth - Differential gene expression	Tucker, 2007
E. coli K12	- Differential gene expression	Vukanti, 2008
E. coli 083:H1	- Increased resistance to thermal and oxidative stress and adhesion to epithelial cells	Allen, 2008
P. aeruginosa PA01	- Increased: biofilm formation; elastase production, and rhamnolipid production; alginate production; resistance to oxidative and thermal stress; Hfq expression - Differential gene expression	Crabbe, 2008 Crabbe, 2010
Streptococcus pneumoniae TIGR4	- Differential gene expression	Allen, 2006
S. aureus N315	- Increased: biofilm formation; susceptibility to whole blood - Decreased: growth; carotenoid production; resistance to oxidative stress; Hfq expression	Castro, 2011
S. aureus RF1, RF6, RF11	- Decreased: carotenoid production; hemolytic activity - Differential gene expression	Rosado, 2010
S. aureus 25923	- Increased: growth and membrane integrity	Vukanti, 2012
Yersina Pestis KIMD27	- Decreased: Hela cell rounding	Lawal, 2010
Haloferax mediterranei	- Increased antibiotic resistance - Differential pigment production and protein expression	Dornmayr-Pfaffenhuemer, 2011
Halococcus dombrowskii	- Decreased cell aggraegation - Differential pigment production and protein expression	Dornmayr-Pfaffenhuemer, 2011
Saccharomyces cerevisiae	- Increased aberrant budding - Differential gene expression	Purevdorj-Gage, 2006
Candida albicans	- Increased: filamentous growth; biofilm formation; antimicrobial resistance - Differential gene expression	Altenburg, 2008 Searles, 2011

LSMMG-cultured S. Typhimurium suffered an increased percent mortality, increased time to death, and required a lower LD50 as compared to control cultures (Nickerson et al. 2000). The success of the flight analog studies using the RWV resulted in the aforementioned two spaceflight experiments involving *S.* Typhimurium. One outcome of these investigations was the documented increased virulence of the bacterium in response to spaceflight, paralleling the bacterium's response to LSMMG as produced by the RWV (Nickerson et al. 2000, Wilson et al. 2007) and validating its use as a spaceflight analog.

In addition to the similarities between *S.* Typhimurium cultured in-flight and within the RWV bioreactor, other commonalities have been demonstrated. For example, scanning electron microscopy images revealed an unidentified extracellular matrix around *S.* Typhimurium cells following spaceflight culture (Wilson et al. 2007), in response to the modeled microgravity conditions within the RWV bioreactor P. *aeruginosa, S. aureus, E. coli*, and *C. albicans* have all demonstrated increased biofilm formation (Lynch et al. 2006, Crabbe et al. 2008, Castro et al. 2011, Searles et al. 2011). With multiple reports of changes in phenotype following exposure to both true microgravity and simulated microgravity, differences in gene expression in response to culture in these environments is not unexpected. What was surprising was the identification of the involvement of Hfq, an RNA chaperone protein that exerts post-transcriptional regulation by binding messenger RNA with small non-coding RNA (Valentin-Hansen et al. 2004), with the mechanism governing the spaceflight response of S. Typhimurium (Wilson et al. 2007). The role for Hfq was validated with the RWV bioreactor and has since been shown to be involved in the modeled microgravity response of both *P. aeruginosa* and *S. aureus* (Crabbe et al. 2010, Castro et al. 2011). While first identified in spaceflight, the use of the RWV bioreactor on Earth produced evidence that suggests that the ability to sense and respond to mechanical stimuli such as microgravity and simulated microgravity may be evolutionarily conserved among structurally diverse prokaryotes.



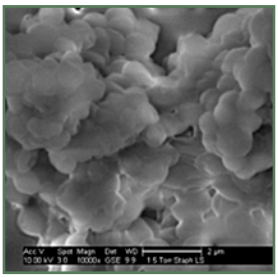


Figure 4. Increased extracellular matrix production as a result of A) spaceflight culture in S. Typhimurium *and modeled microgravity in B)* P. aeruginosa *and in C)* S. aureus *(Wilson et al. 2007, Crabbe et al. 2008, Castro et al. 2011).*

What Should Principal Investigators Know About Conducting Research on the ISS?

Supporting research in science and technology is an important part of NASA's overall mission. NASA solicits research through the release of NASA Research Announcements (NRA), which cover a wide range of scientific disciplines. All NRA solicitations are facilitated through the web-based NASA Solicitation and Proposal Integrated Review and Evaluation System (NSPIRES) http://nspires.nasaprs.com/external/. Registering with NSPIRES allows investigators to stay informed of newly released NRAs and enables submission of proposals. NSPIRES supports the entire lifecycle of NASA research solicitations and awards, from the release of new research calls through the peer review and selection process.

In planning the scope of their proposal, investigators should be aware of available resources and the general direction guiding NASA research selection. NASA places high priority on recommendations from the 2011 National Research Council's NRC Decadal Survey, which placed emphasis on hypothesis-driven spaceflight research. In addition, principal investigators (PI) should be aware that spaceflight experiments may be limited by a combination of power, crew time, or volume constraints. Launch and/or landing scrubs are not uncommon, and alternative implementation scenarios should be considered in order to reduce the risk from these scrubs. Preliminary investigations using ground-based simulators may be necessary to optimize procedures before spaceflight. Also, many experiments require unique hardware to meet the needs of the spaceflight experiment. To understand previous spaceflight studies, prospective PIs should familiarize themselves with the NASA ISS Program Science Office database, which discusses research previously conducted on the ISS, including that of the International Partners. A detailed catalog of previous, current, and proposed experiments, facilities, and results, including investigator information, research summaries, operations, hardware information, and related publications is available at www.nasa.gov/iss-science through the NASA ISS Program Office. Additionally, details pertaining to research previously supported by the Space Life and Physical Sciences Research and Applications Division of NASA's Human Exploration and Operations Mission Directorate can be located in the Space Life & Physical Sciences Research and Applications Division Task Book in a searchable online database format at: https://taskbook.nasaprs.com/Publication/welcome.cfm.

When planning microbiology experiments bound for the ISS, it is important that PIs understand the exposure risks to the crew members and implement the required levels of containment. Only microorganisms with a biosafety level of 1 or 2 are allowed to be flown to the ISS. Biosafety level 1 organisms usually require only one level of containment. Biosafety level 2 organisms are broken into two categories, those that are moderate risk agents associated with human diseases and in which primary exposure routes include percutaneous exposure, ingestion, and mucous membrane exposure. Microorganisms that meet this description generally require two levels of containment. Biosafety level 2 organisms that are associated with a higher risk of human diseases in which a lower infectious dose, the likelihood of aerosolization, and/or larger amounts of agent are present may require three levels of containment. In order to fly any biological sample, an investigator must submit a biohazardous materials form through the NASA Biosafety Review Board (BRB). To register with NASA's BRB, obtain more information on flying biohazardous samples, and for the necessary form(s) visit: https://microbrb.jsc.nasa.gov/public/.

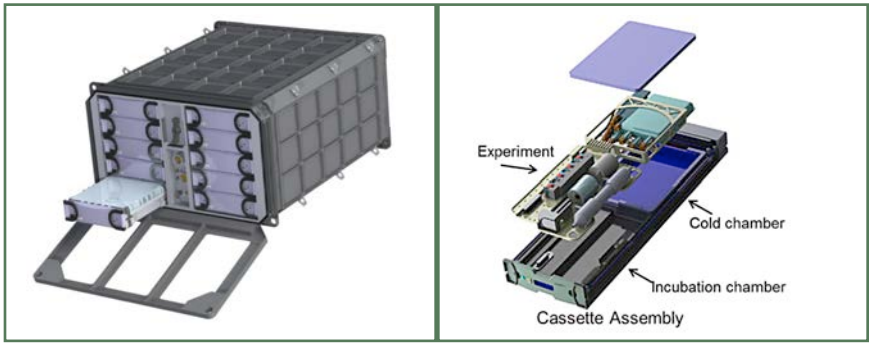
Multipurpose Facilities Available on the ISS

Biological Experiment Laboratory (BioLab): Biolab Includes an incubator, microscope, spectrophotometer, glovebox, freezer units, and two centrifuges to simulate the effects of gravity. A variety of hardware existing inserts and containers for microbiological experimentation are available through the primary European Space Agency (ESA) contractor Astrium (www.astrium.eads.net).

Artist's impression of BioLab. Credit ESA

Bioculture System (BIOS): The NASA Bioculture System is an advanced space bioscience culturing system capable of supporting variable duration and long duration experiments on the ISS. BIOS provides the ability to culture mammalian and non-mammalian cells and will allow for investigations into host-pathogen interactions.

BIOS has 10 independent incubation chambers and allows for automated sampling and injection timelines.



BIOS Culture System

Astronaut Michael E. Lopez-Alegria, Expedition 14 commander and NASA space station science officer, replaces the European Modular Cultivation System (EMCS) Experiment Container (EC) in the Destiny laboratory of the International Space Station.

European Drawer Rack (EDR): EDR supports seven Experiment Modules (EMs), each with independent cooling, power, and data communications as well as vacuum, venting, and nitrogen supply, if required.

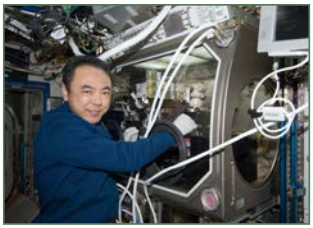
European Modular Cultivation System (EMCS): EMCS allows for cultivation and stimulation of biological experiments under controlled environmental conditions (e.g. temperature, gas, water supply and light). The EMCS has two centrifuges that can spin at 0 to 2 times Earth's gravity.

EXpedite the PRocessing of Experiments for Space Station Racks (EXPRESS Racks): EXPRESS Racks is a multipurpose payload rack systems that provide structural interfaces, power, data, cooling, water, and other items needed to operate microbiological experiments in space.

The image shows a front view of EXpedite the PRocessing of Experiments to Space Station EXPRESS Rack 4 in the U.S. Laboratory, Destiny, during Expedition 9.

Image Processing Unit (IPU): The IPU receives, records, and downlinks experiment image data for experiment processing. The IPU is housed in the Ryutai (fluid) experiment rack.

Light Microscopy Module (LMM)



Japan Aerospace Exploration Agency astronaut Satoshi Furukawa, Expedition 29 flight engineer, works at the Microgravity Science Glovebox (MSG) in the Destiny laboratory of the International Space Station.

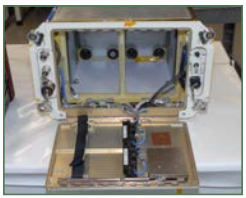
Greg Chamitoff, Flight Engineer - 2 works with the Saibo facility in the Japanese Experiment Module, Kibo. Photo was taken during Expedition 17.

Light Microscopy Module (LMM): The light imaging microscope that takes digital images and videos across many levels of magnification using standard Leica objective lenses. It is capable of high resolution black and white microscopy, bright field, epifluorescent and fluorescent techniques.

Microgravity Science Glovebox (MSG): The MSG is a contained work environment for research with liquids and hazardous materials. It is equipped with a front window, built-in gloves, video system and data downlinks allow for monitoring enclosed experiments from the ground.

NanoRacks: Nanoracks contains optical and reflective microscopes with digital image retrieval for ISS experiments. A NanoRacks Plate Reader is also available to monitor samples in microtiter plates with 96 wells with controls for temperature and stirring.

Saibo Rack: Saibo rack contains the Cell Biology Experiment Facility (CBEF) and Clean Bench (CB). CBEF is an incubator with a micro-G compartment and a1G compartment equipped with small centrifuge. CB is a glovebox with a HEPA filter and high-performance optical microscope.



Advanced Biological Research System (ABRS)

Hardware Available for Microbial Experiments

Advanced Biological Research System (ABRS): ABRS is a modular environmental chamber with two growth chambers, each capable of independently controlling temperature, illumination and atmospheric composition for growing and monitoring microbes.

Biorisk: Biorisk is a suite of hardware (Biorisk-MSV container; Biorisk-KM case; Biorisk-MSN) used to monitor the impact of the space environment on bacteria and fungi.

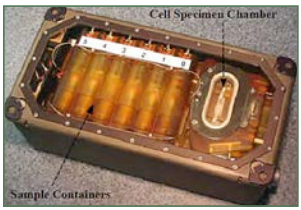
Biological Research in Canisters (BRIC)

Biological Research in Canisters (BRIC): A BRIC is an anodized-aluminum cylinder used to provide passive stowage for investigations studying the effects of space flight on microbes. It includes fluid chambers for controlled nutrient supply and sample fixation. BRIC-Opti hardware provides a closed environment with an atmosphere of known initial composition for microbial growth experiments.

Cell Culturing (CellCult): The CellCult is an automated cell culture container with one rotating reactor vessel that is fed fresh medium from a nutrient bag in perfusion, batch, or sampling mode.

Expose: Expose provides short- and long-term exposure of microbes to space conditions and solar UV radiation. It is installed outside the ISS at the Russian Zvezda service module (Expose-R) or European Columbus laboratory (Expose-E).

Fluid Processing Cassette (FPC): The FPC provides a triple-containment system used for feeding and fixing microbial culture experiments. The FPC is capable of autonomous and battery-powered operations.



Single Loop Cell Culture (SLCC)

Single Loop Cell Culture (SLCC): The SLCC system can be used for microbial growth, sub-culturing and sampling. It uses active perfusion flow to provide nutrients and gas exchange and to purge waste products into bladder tank.

JAXA Particle Counter: The Particle Counter is designed to detect particles floating in air. It is able to display the numeral of particles for six size ranges: >0.5, >1.0, >2.0, >3.0, >5.0, >10.0 micro meters. The Particle Counter operates with four Alkaline D batteries.

Hardware Available for Incubation and Storage

Photograph of CGBA during Increment 33 showing open containment volume and sample canisters. Image courtesy of NASA.

Commercial Generic Bioprocessing Apparatus (CGBA): The CGBA is a programmable, accurate temperature-controlled system for cold stowage or incubation studies. The BioServe Culture Apparatus (BCA) inside the CGBA was developed for suspension cell culture research. The hardware allows for passive gas exchange in a sterile environment and can provide growth media, time-course sampling and fixation of cultures.

General Laboratory Active Cryogenic ISS Equipment Refrigerator (GLACIER): GLACIER is an ultra-cold freezer (capable of temperatures down to -165°C) with a storage volume of 1.35L.

Kriogem-3M: Kriogem-3M is a refrigerator used for the stowage of biological samples and incubation of certain types of bioreactors.

Kubik with centrifuge configuration loaded with experiment containers. Image courtesy of ESA.

KUBIK: KUBIK is a portable incubator that can function as a growth chamber (+ 6 to 38°C) or stowage. It is equipped with compartments for microgravity or artificial gravity using a centrifuge.

Minus Eighty-Degree Laboratory Freezer (MELFI): MELFI is a refrigerator/freezer (capable of temperatures +4°C to -80°C) with a storage volume of 175L.

ISS Commander Sunita Williams and Aki Hoshide transferring MELFI samples during Expedition 33.

Microgravity Experiment Research Locker/Incubator (MERLIN): MERLIN is a multipurpose freezer, refrigerator or incubator with temperatures between -20°C and 48.5°C with a storage volume of 4.17L.

Sample Collection, Handling, and Fixation Devices Available on the ISS

Biotube: A large volume incubator with triple-containment and environmental controls capable of delivering fixative to specimens and remote monitoring through an integrated digital imaging system.

Kennedy Space Center Fixation Tube (KFT)

Kennedy Space Center Fixation Tube (KFT): KFT was designed to contain microbial samples during flight with three safety levels of containment. Samples can be chemically fixed or stained by activating fluids stored inside a separate tube chamber.

Portable Glovebox (PGB): The PGB is a modular system for preventing contamination of microbes when other experimental hardware is opened for observations, sampling, or fixations.

Wet Lab Kit: This is a customizable kit with consumables and tools for supporting in-orbit sample processing (e.g., disposable glove bags, swabs, wet wipes, etc.).

JAXA Sampling Kit: JAXA Sampling Kit (Swab & Tube, Sampling Sheet, Microbial Detection sheet(MDS)) is used to collect and detect microbes. Sampling Sheet is an adhesive sheet to collect microbes. MDS is a sheet-type culture medium with non-woven fabrics, which detects microbes on the ISS.

Process for Payload Development and Implementation

Following selection of an experiment for spaceflight, the PI will work with a payload integrator or hardware developer to define the most suitable hardware, and determine if hardware needs to be created or modified. The research team in combination with payload integrations will establish the specific laboratory requirements needed to support the experiment. Through these collaborative efforts, concerns such as crew procedures and crew training, the need for spare parts and/or contingencies involving hardware, and stowage requirements of the samples will be addressed and resolved. It is highly recommended that the PI preform a series of investigations using the identical hardware and under configuration and control conditions similar to those anticipated in-flight prior to the launch. This will prevent unforeseen issues with the hardware and allow specific mission constraints to be defined, and mitigated, prior to the experiments implementation once aboard the ISS. It is also within this

time frame that the science team needs to characterize the details involved with their synchronous ground controls. The PI's team should also have finalized all post-landing procedures, including sample preservation, storage, and transport, and data acquisition prior to the launch.

Another option to flying your experiment is through the Center for the Advancement of Science in Space (CASIS) (http://www.iss-casis.org). CASIS is a nonprofit organization tasked by U.S. Congress and NASA with promoting and enabling research on ISS. CASIS can be used for all stages of payload development and can match PIs with implementation partners (table below) who can provide heritage hardware or new flight packages:

Table 3. Implementation Partners for Flight Experiments on the ISS

Company	Contact Information
The Aerospace Corporation	www.aero.org
Astrium North America	www.astrium-na.com
Astrotech Corporation	www.astrotechcorp.com
Aurora Flight Sciences	www.aurora.aero
Bionetics Corporation	www.bionetics.com
Bioserve	www.colorado.edu/engineering/BioServe
Boeing	www.boeing.com
CSS-Dynamac	www.css-dynamac.com
Hamilton Sundstrand	www.hamiltonsundstrand.com
Jamss America	www.jamssamerica.com
Kentucky Space, LLC	www.kentuckyspace.com
MDA	www.mdacorporation.com
MEI Technologies	www.meitechinc.com
Nanoracks LLC	www.nanoracks.com
Orbital Technologies Corporation	www.orbitec.com
Paragon TEC	www.paragontec.net
Qinetiq	www.qinetiq-na.com
Space Systems Concepts, Inc.	www.space-concepts.com
Space Systems Research Corporation	www.spacesystemsresearch.com
Tec-Masters, Inc.	www.tecmasters.com
Techshot	www.techshot.com

Table 3. Implementation Partners for Flight Experiments on the ISS *continued...*

Company	Contact Information
Teledyne Brown Engineering, Inc.	www.tbe.com
Thales Alenia Space	www.thalesgroup.com/space
UAB	www.uab.edu/cbse
Wyle Integrated Science and Engineering	www.wyle.com
Zin Technologies	www.zin-tech.com

Funding Opportunities and Points of Contact

There are various avenues that can result in funding for research to be conducted on the ISS, and the source of funding often dictates the availability of launch opportunities. Generally, funding for microbiology-related research is awarded through NASA-sponsored research announcements (NRA's), ISS National Laboratory awards through other government agencies, private commercial enterprise, nonprofit organizations, and research awards sponsored by the ISS International Partners. It is not the responsibility of a researcher awarded an ISS flight experiment to fund costs associated with launch or the ISS laboratory facilities. Greater detail concerning current funding opportunities for ISS research can be found through the NASA ISS research website http://www.nasa.gov/mission_pages/station/research/ops/research_information.html.

The NASA Solicitation and Proposed Integrated Review and Evaluation System (NSPIRES) can be accessed via http://nspires.nasaprs.com/external/.

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Acronyms

ABRS	Advanced Biological Research System
ADVASC-SS	Advanced Astroculture Support System
ARCTIC	Advanced Thermoelectric Refrigerator/Freezer
BCA	BioServe Culture Apparatus
BIOS	Bioculture System
BRB	Biosafety Review Board
BRIC	Biological Research in Canisters
BSTC	Biotechnology Specimen Temperature Controller
CASIS	Center for the Advancement of Science in Space
CB	Clean Bench
CBEF	Cell Biology Experiment Facility
CBOSS	Cellular Biotechnology Operations Support Systems
CGBA	Commercial Generic Bioprocessing Apparatus
EC	Experiment Container
EDR	European Drawer Rack
EM	Experiment Module
EMCS	European Modular Cultivation System
ESA	European Space Agency
EXPRESS	EXpedite the PRocessing of Experiments for Space Station
FPC	Fluid Processing Cassette
GLACIER	General Laboratory Active Cryogenic ISS Equipment Refrigerator
GSM	Gas Supply Module
IPU	Image Processing Unit
ISS	International Space Station
JAXA	Japan Aerospace Exploration Agency
KFT	Kennedy Space Center Fixation Tube
LMM	Light Microscopy Module
LSMMG	Low-Shear Modeled Microgravity
MDS	Microbial Detection Sheet
MELFI	Minus Eighty-Degree Laboratory Freezer
MERLIN	Microgravity Experiment Research Locker/Incubator
MIC	Minimal Inhibitory Concentration
MSG	Microgravity Science Glovebox
NRA	NASA Research Announcements
NRC	National Research Council
NSF	National Science Foundation
NSPIRES	NASA Solicitation and Proposal Integrated Review and Evaluation System
PGB	Portable Glovebox
PI	Principal Investigators
RWV	Rotating-Wall Vessel
SAMS	Space Acceleration Measurement System
SLCC	Single Loop Cell Culture
USSR	Union of Soviet Socialist Republics

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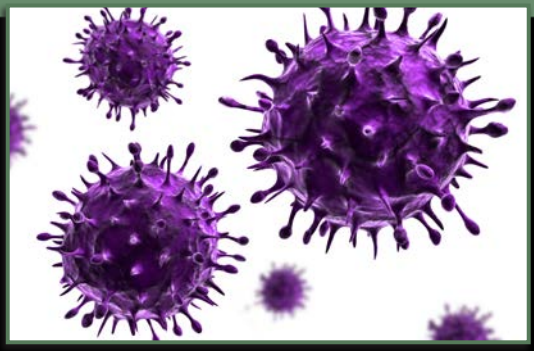

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