

Fiscal Year:	FY 2018	Task Last Updated:	FY 01/31/2019
PI Name:	Lorenzi, Hernan Ph.D.		
Project Title:	Study of the Impact of Long-term Space Travel on the Astronaut's Microbiome		
Division Name:	Human Research		
Program/Discipline:	HUMAN RESEARCH		
Program/Discipline-- Element/Subdiscipline:			
Joint Agency Name:	TechPort:	No	
Human Research Program Elements:	(1) HHC: Human Health Countermeasures		
Human Research Program Risks:	(1) Medical Conditions: Risk of Adverse Health Outcomes and Decrements in Performance Due to Medical Conditions that occur in Mission, as well as Long Term Health Outcomes Due to Mission Exposures (2) Microhost: Risk of Adverse Health Effects Due to Host-Microorganism Interactions		
Space Biology Element:	None		
Space Biology Cross-Element Discipline:	None		
Space Biology Special Category:	None		
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Comments:			
Project Type:	Flight	Solicitation / Funding Source:	2010 Crew Health NNJ10ZSA003N
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No. of Post Docs:	1	No. of PhD Degrees:	0
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No. of Master's Candidates:	0	No. of Bachelor's Degrees:	0
No. of Bachelor's Candidates:	0	Monitoring Center:	NASA ARC
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Flight Program:	ISS		
Flight Assignment:	ISS NOTE: Extended to 9/30/2018 per NSSC information (Ed., 11/21/17) NOTE: Element change to Human Health Countermeasures; previously Space Human Factors & Habitability (Ed., 1/18/17) NOTE: Extended to 9/30/2017 per F. Hernandez/ARC (Ed., 3/11/16) NOTE: Extended to 9/30/2016 per A. Chu/ARC (Ed., 8/5/14) NOTE: Gap changes per IRP Rev E (Ed., 3/19/14)		
Key Personnel Changes/Previous PI:	Drs. Mark Ott and Duane Pierson are collaborators on this project. March 2016: Added Research Assistant Kelvin Moncera as key personnel to the study. February 2015: Added Key personnel Postdoctoral fellow Alexander Voorhies and Dr. Karen Nelson to the study. July 2014: Manolito Torralba and Dr. Satish Mehta have been incorporated as key personnel. August 2012: Scott Peterson (former co-PI of the project) and Shannon Williamson (key personnel) are not participating in this project any more.		

COI Name (Institution):	Pierson, Duane Ph.D. (NASA Johnson Space Center) Ott, Charlie Mark Ph.D. (NASA Johnson Space Center)
Grant/Contract No.:	NNX12AB02G
Performance Goal No.:	
Performance Goal Text:	
Task Description:	<p>Our goal is to determine how the composition of the human microbiome changes during long-term space exploration and to evaluate its potential impact on astronauts' health. Some microbial species from the human microbiome have a beneficial or protective effect on health; the loss of these species can lead to an altered metabolic function and, in conjunction with reduced immune response, may increase the chance of infection by opportunistic pathogens. In our proposal we will elaborate the notion of the microbiome as harbingers or sentinels to monitor a variety of aspects of the human host, including associations with health status, environmental stress, and exposure to space conditions. By sampling the microbiome of astronauts on Earth while in peak physical health and during subsequent times of stress, including long-term exposure to microgravity, g-forces, radiation, and changes in health status, we will be able to define signatures of human response to a variety of relevant aspects of space travel. We propose to characterize the bacterial and viral microbiome from various body sites of up to nine astronauts who travel to space at several time points before, during, and after a space mission. Also we will assess the astronauts' immune function before, during, and after the mission by analyzing their collected saliva samples for reactivated latent viruses and cortisol levels, two indicators commonly evaluated during spaceflight immune and stress studies and cytokines from blood samples. Finally, we will correlate the collected microbiome and immune function data with other measured metadata including astronaut health and hygiene as well as environmental factors such as temperature, humidity, and environmental microbial samples that will be collected, depending upon availability, from various surfaces on the International Space Station (ISS).</p>
Rationale for HRP Directed Research:	
Research Impact/Earth Benefits:	<p>The results of this study will provide insights into how the microbial population of the environment affects the composition and dynamics of the human microbiome. This is relevant to studies of respiratory diseases such as asthma and allergies.</p> <p>Investigating the impact of stress and status of the immune system on the human microbiome, and potentially on human health, during a space mission is also applicable to equivalent stressful situations on Earth. Some of the conclusions of this project will also be useful in situations where a group of individuals are confined in a relatively small and closed space for a long period of time, such as a submarine crew.</p>
	<p>FINAL REPORTING FEBRUARY 2019:</p> <p>1) Sample collection and processing.</p> <p>During this period all samples have been already collected and processed.</p> <p>Characterization of the overall spatial patterns of microbial community structure showed that the factor that most contributed to variation among all samples was sampling site, in agreement with previous studies showing that the skin and nose microbiomes are more similar to each other than to those from feces or mouth. In addition, samples collected from the same body site tended to cluster by crew member. Interestingly, ISS environmental samples overlapped with specimens from the two skin sites and the nose microbiota. Further analysis showed that there was no significant difference among bacterial species that were present/absent in ISS microbial communities and inflight forehead and forearm skin microbiomes.</p> <p>2) Changes in beta diversity of the astronauts' microbiota.</p> <p>To determine the influence that the amount of time spent at ISS has in any observed changes in astronaut microbiomes, we compared microbial profiles of microbiome samples collected at every inflight and postflight time points to all preflight time points (as a baseline). This analysis showed that compositional changes of the nose, skin, and gastrointestinal (GI) microbiomes were rapid and became evident by FD7. These changes persisted for at least six months until the end of the mission at the ISS. Furthermore, beta diversity changes did not significantly increase with the time astronauts spent in space, although preflight-inflight dissimilarity distances of the two skin sites and the nose microbiota showed a very modest upward trend associated with time spent inflight. In addition, compositional shifts in the skin and nose microbiomes persisted for at least 60 days after the astronauts returned to Earth. However, the composition of the GI microbiota became similar to preflight samples within two months of the astronauts' return from the ISS.</p> <p>3) Alteration of the microbial composition of the crew microbiome associated with the space environment.</p> <p>To investigate the influence of the ISS as a contained and human built environment on the crew members' microbiomes, differential abundance analysis was performed between samples collected before, during, and after the mission to the ISS. This analysis identified 15 gastrointestinal genera whose abundance significantly changed in space. Ten out of the 15 genera belonged to the phylum Firmicutes and most belonged to the order Clostridiales. Among these taxonomic groups, there was a more than five-fold inflight reduction in Akkermansia and Ruminococcus, and a ~3-fold drop in Pseudobutyrvibrio and Fusicatenibacter. Most of these compositional changes reverted to preflight levels after astronauts returned to Earth, with the exemption of four genera of the phylum Firmicutes. Examination of skin samples also revealed changes in the relative abundance of several bacterial groups corresponding to 43 and 31 genera in the forearm and forehead, respectively. Noteworthy, skin microbial communities whose abundance decreased in space were mostly Gram-negative Proteobacteria. These groups included bacteria from the genus Acinetobacter, Cloacibacterium, and Pseudomonas. In contrast, most of the skin bacteria that became more abundant inflight belonged to the phylum Firmicutes, Bacteroidetes, and Actinobacteria, including bacteria of the genus Streptococcus, Staphylococcus, and Corynebacterium. Postflight samples showed a similar trend as inflight samples, with lower Proteobacteria and higher Firmicutes, Bacteroidetes, and Actinobacteria compared to Preflight skin. In addition, we observed a similar but milder response of the nares microbiota to the space environment with a significant drop in three genera of Gram-negative Betaproteobacteria, all of which were also reduced in skin. Likewise, nose inflight samples showed increases in five</p>

Gram-positive genera that also became more abundant on the skin of astronauts inflight. Many of these changes dissipated after the astronauts returned to Earth.

4) Identification of microbial changes associated with Astronauts' immune dysregulation in space.

To gain insights into the potential impact of changes to the microbiome during spaceflight on immune functioning, changes to cytokine abundance in plasma were compared to changes in the composition of the GI microbiome. This analysis identified strong evidence of association between changes in astronauts' GI microbiome and changes in cytokine profiles. Most notably, the abundance of bacteria of the genus *Fusicatenibacter* was negatively correlated with the concentration of pro-inflammatory cytokines IL-8, IL-1b, IL4, and TNFa. In addition, changes in bacteria of the genus *Dorea* were also negatively correlated with changes in the level of several cytokines including IL-1b, IL-1ra VEGF, and MIP-1b, all of which were increased in space.

5) ISS environment and its interaction with the Astronauts Microbiome.

Given that the ISS microbiota resembled that of the astronauts' skin (see above), we hypothesized temporal changes could be influenced by the arrival of new crew members to the ISS every three months. To test this hypothesis, we compared the microbial composition of the ISS samples from early and late time points to the skin microbiome of astronauts that traveled to the ISS at the beginning or end of the study. This analysis showed that while there were no differences in the species present/absent between skin and ISS samples at either early or late time points, early skin microbiome samples had a significantly different composition to late ISS microbiome samples and vice versa. Moreover, comparative analysis of taxonomic profiles showed that the skin and ISS samples collected by the time astronauts were leaving the ISS were more similar to each other than the ISS and skin samples collected at FD7, when crew members had just arrived at the ISS. Comparison of differences in microbial alpha diversity and richness revealed that the six ISS sites surveyed had similar richness and Shannon diversity values. Alpha diversity and richness were also similar across environmental and inflight skin samples. However, we found that alpha diversity and richness of ISS samples significantly fluctuated over time in a manner that correlated with changes in inflight alpha diversity and richness of the crew skin microbiota. In spite of the fluctuations in the composition of the ISS microbial communities over time, each of the assessed ISS surfaces had a small proportion of OTUs (< 4%) that were highly prevalent during the entire duration of our study, most of them from the phyla Firmicutes, Actinobacteria, and Proteobacteria, and therefore, may be long-term residents of the ISS environment.

6) Astronauts' virus reactivation and hormone stress levels.

Reactivation and shedding of latent varicella zoster virus (VZV), Epstein bar virus (EBV), and Herpes simplex virus (HSV1) as well as diurnal salivary cortisol, alpha-amylase, and dehydroepiandrosterone (DHEA) were measured prospectively in 10 astronauts before, during, and after their mission at the ISS to assess astronauts' stress. Two astronauts did not shed any virus in any of their samples collected during the study. VZV reactivation was detected in the saliva of four crew members. Except for one crew member that showed VZV reactivation by L-60, no VZV was detected in saliva before flight. However, VZV was significantly reactivated in space, reaching a peak by FD90. After 30 days of the return to Earth, three of the astronauts became negative for VZV except for one astronaut that remained positive for up to 180 days. EBV and HSV1 were detected in the saliva of three and five astronauts, respectively, but no association was found between detection in saliva and spaceflight. No significant changes in levels of salivary cortisol were detected during the entire mission in any of the crew members. The inflight salivary concentration of alpha-amylase, however, was higher than preflight values reaching a maximum by the end of the mission in the ISS. DHEA showed an opposite trend, becoming less abundant by FD90 at the ISS.

7) Changes in the metabolic capacity of the GI microbiome during space travel.

To investigate whether the observed changes in the microbial composition of the astronauts' GI microbiome have any consequence on its metabolic capacity we carried out metagenomic sequence analysis of the GI microbiome of six crew members during their missions to the ISS. Comparative analysis of the enzyme profiles predicted for each astronaut metagenome showed that, within subjects, inflight enzyme profiles tend to be different from preflight or postflight samples. Further analysis at the individual enzyme level identified 10 enzymatic functions that were differentially abundant between pre and inflight metagenomes, while only EC3.7.1.3 changed and became less abundant in postflight samples compared with preflight samples. In addition, we identified five metabolic pathways that were either over or underrepresented in inflight samples of the GI microbiome. One of these pathways, which consistently increased in space, corresponded to the pathway for the biosynthesis of polysaccharides, or lipopolysaccharides (LPS), which is a typical component of the outer membrane of gram negative bacteria. Taxonomic analysis of the genes participating in the LPS pathway showed that the observed changes are mostly driven by changes in the abundance of bacteria from the genus *Bacteroides*. The direction of the changes in gene abundance in the other four pathways identified were less consistent than the LPS pathway, with different GI metagenomes responding differently to space travel. One of these pathways was the one responsible for the biosynthesis of the bacterial flagellum. GI bacteria encoding for this pathway were enriched in three crew members and reduced in the metagenomes of the other three astronauts analyzed. Further taxonomic analysis showed that for all astronauts changes in this pathway were mostly caused by increases or decreases of bacteria of the genus *Eubacterium* and *Roseburia*, two of the most abundant motile bacteria found in the GI tract of healthy individuals. In agreement, the abundance of genes involved in bacterial chemotaxis was found to significantly change during spaceflight in a fashion similar to the related bacterial flagellum biosynthesis pathway. Further taxonomic analysis showed that the changes were mainly driven by alterations in the relative abundance of bacteria from the genus *Eubacterium* and *Roseburia*. Also, we investigated how changes in the bacterial communities of the GI tract affected specific bacterial metabolic pathways that are relevant to human health. In particular, we assessed pathways involved in the biosynthesis of short chain fatty acids (SCFA) butyrate and propionate and of vitamins B1, B6, Biotin, and Folate. None of these pathways seemed to be significantly perturbed in space. However, two astronauts did show a reduction in space of some genes encoding for key enzymes relevant for the biosynthesis of vitamin B1 (EC2.5.1.3 and EC2.7.4.7).

ANNUAL REPORTING AUGUST 2017:

1 - Inform Consent Briefings, Recruitment of Astronauts, and Base Data Collection

We have recruited all 9 subjects requested for the study plus one backup volunteer and inform consents have been already signed by all of them. All preflight, inflight, and postflight samples required for the project have been already collected and are at different stages of the processing pipeline. All swab, stool, and water samples were delivered to J

Task Progress:

Craig Venter Institute (JCVI) for 16S and metagenomic sequencing and analysis. Saliva and Blood samples were sent to Dr. Ott's laboratory for measurement of cytokines, virus reactivation, and cortisol levels in blood and saliva samples, respectively. Sample sets from each astronaut are being processed altogether to reduce variation due to methodological error.

2 Sequencing and analysis of 16S rRNA gene taxonomic profiles.

2.1. Sample processing.

During the current period we have finalized the sequencing and analysis of 16S taxonomic profiles for the entire set of swab and stool samples collected from Astronauts A to I, totaling 507 samples (88 forehead, forearm, nose, and tongue swab samples, 73 stool samples, and 82 ISS environmental swab samples).

2.2 Analysis of alpha diversity across all sampled sites.

It has been shown that changes in the microbial diversity of the gastrointestinal (GI) tract is associated with a number of human diseases. In addition, previous studies on culturable bacteria have shown that diversity of GI bacteria drops after a space mission. Therefore, we investigated how bacterial diversity of the five Astronauts' microbiomes surveyed was affected by space-travel.

This analysis showed that in general the diversity of the GI microbiome significantly increased in space, contrary to what it was expected based on previous results on culturable bacteria. Our analysis also showed that gut bacterial diversity went back to its original preflight levels after the crew returned to Earth. These results underline the importance of collecting microbiome samples in space, given that differences in alpha diversity between pre and inflight stool samples were mostly erased once study subjects returned to Earth.

Alpha diversity from the two skin sites, forehead and forearm, significantly changed in space, although in this case the direction of the change was not as consistent as in stool samples. In general, the diversity of the forehead skin microbiota did not return to their preflight levels within 60 days after landing. Noteworthy, unlike the gut microbiome, alpha diversity of the nose microbiota tended to be lower in space and did not recover after flight. It is possible that the reduction in inflight alpha diversity is a consequence of the highly clean environment of the ISS.

The tongue microbiota is the only crew microbiome surveyed that did not show any overall significant changes during the entire mission. However, at the individual level, there were some exceptions.

2.3 Analysis of changes in the taxonomic profiles of the astronauts' microbiome.

Next we investigated how the taxonomic profiles of the astronauts' microbiome changed during a mission to the ISS. In all cases there was a significant change in the composition of the gut microbiota in space, but recovery of the gastrointestinal microbiome after flight was dissimilar among astronauts. A similar analysis on the nose microbiota showed that, in general, the composition of the nose microbiome did not significantly change in space, although a slight change was detected between postflight samples and either inflight and preflight samples.

Contrary to the nose microbiota, both skin microbiomes surveyed showed a strong compositional change in space that did not recover after the crew returned to Earth. This result suggests that the skin microbiota is more sensitive to environmental changes compared to the other three microbiomes studied. One possible explanation is that the skin surface is more exposed to external conditions than the nose, tongue, and gut, making the skin microbiota more susceptible to changes in the environmental conditions.

Lastly, the composition of the tongue microbiome showed a slight change in space, but most of those changes reverted to their original preflight composition by R+60.

2.4 Study of the ISS environmental microbiota.

To study the stability of the ISS microbiome over time, we collected samples from six different sites on the ISS over a period of about three years. This analysis showed that the diversity of the ISS environmental microbiota is relatively stable over time, with some sites being more variable than others. This difference in microbial stability might be caused by the fact that some sites in the ISS are cleaned more frequently than others or are accessed by different crew members.

Study of the microbial composition of the ISS environment also showed that the microbes from the air filter inlet sampled in this study were different from those microorganisms inhabiting the rest of the ISS sites surveyed. This analysis also revealed that the composition of the ISS environmental microbiota significantly change over time and that those changes are likely to be driven by the skin microbiota of the crew.

3 Changes in cytokine concentration in plasma.

During this period we have also finalized with the cytokine analysis of plasma samples. This analysis revealed a number of cytokines whose concentration changed in space, most of them associated with the inflammatory response. These results are in line with previous studies on the same topic.

4. Future directions.

We are currently working on the integration of metagenomic-based microbiome functional data with cytokine profiles, astronauts' stress information, and additional metadata collected for this project. Given the importance of the human GI microbiome on human health we are also generating 16S taxonomic profiles from the backup crew member to increase the significance of the results obtained so far.

Bibliography Type:

Description: (Last Updated: 04/10/2021)

Abstracts for Journals and Proceedings

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Awards	Lorenzi H. "PI's research article 'Study of the impact of long-duration space missions at the International Space Station on the astronaut microbiome' was awarded by Scientific Reports – Nature – as one of the Top 100 Scientific Reports microbiology papers in 2019. The paper was among the top 11 papers downloaded during 2019 (actually it was 27th). Citation: https://www.nature.com/articles/s41598-019-46303-8 " Mar-2020