Fiscal Year:	FY 2016	**Task Last Updated:**	FY 08/03/2015
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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Organization Name:	National Institute of Health		
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City:	Bethesda	**State:**	MD
Zip Code:	20892	**Congressional District:**	8
Comments:			
Project Type:	Flight	**Solicitation / Funding Source:**	2010 Crew Health NNJ10ZSA003N
Start Date:	10/01/2011	**End Date:**	09/30/2017
No. of Post Docs:	1	**No. of PhD Degrees:**	0
No. of PhD Candidates:	0	**No. of Master' Degrees:**	0
No. of Master's Candidates:	0	**No. of Bachelor's Degrees:**	0
No. of Bachelor's Candidates:	0	**Monitoring Center:**	NASA ARC
Contact Monitor:	Griko, Yuri	**Contact Phone:**	650-604-0519
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Flight Program:	ISS		
Flight Assignment:	ISS 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:	August 2012: Scott Peterson (former co-PI of the project) and Shannon Williamson (key personnel) are not participating in this project any more. Drs. Mark Ott and Duane Pierson are collaborators on this project. July 2014: Manolito Torralba and Dr. Satish Mehta have been incorporated as key personnel. February 2015: Added Key personnel Postdoctoral fellow Alexander Voorhies and Dr. Karen Nelson to the study.		
COI Name (Institution):	Pierson, Duane (Johnson Space Center) Ott, Charlie (Johnson Space Center)		
Grant/Contract No.:	NNX12AB02G		
Performance Goal No.:			

Performance Goal Text:	
Task Description:	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).
Rationale for HRP Directed Research:	
Research Impact/Earth Benefits:	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. 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.
Task Progress:	1. Base Data Collection Collection of pre-flight, in-flight, and post-flight samples started on February 2013 and approximately two thirds of the projected samples are already collected and delivered to JCVI (J Craig Venter Institute) or the Johnson Space Center (JSC) for sample processing and analysis. 2. Preliminary analysis of 16S rRNA gene taxonomic profiles. 2.1 Global comparative analysis of phylogenetic profiles across sites. To investigate the composition of the microbial communities present in the samples recollected by the astronauts we amplified and sequenced the V4 variable region from the bacterial 16S rRNA gene by PCR from total DNA extracted from 156 participant swab samples (37 forehead, 36 forearm, 41 nares, and 42 tongue), 31 fecal samples, and 30 ISS environmental samples (27 from different surfaces and 3 from the water tank). Then PCR amplicons were sequenced in an Illumina MiSeq machine and sequencing data was used to identify the bacterial species that were present in each of the samples. All samples analyzed in this report corresponded to baseline pre-flight (L-240, L-150, L-90, and L-60), in-flight (FD7, FD90, and R-14), and post-flight (R+1, R+30, and R+60) time points. Preliminary analysis of the 16S sequencing data from the 187 human samples described above demonstrated that our data were consistent with previous human microbiome studies and findings from the Human Microbiome Project. Indeed, our analysis showed that the main source of compositional variation across microbial communities was the isolation site. Our results are also in agreement with previous studies showing that the skin and nose microbiomes were more similar to each other than to those from feces or tongue [1,2]. In addition, for samples derived from the same body site, those collected from the same participant were more similar to each other than those coming from different subjects, except for forearm samples (stool $p < 0.001$, forehead p-value = 0.027, nose p-value < 0.001, tongue p-value < 0.001). Similarly, microbial composition analysis of 30 environmental microbial samples collected at different time points from the ISS also clustered by sampling site based on their beta diversity values ($p < 0.001$). Beta diversity is a measurement of the proportion of species shared by two samples. Interestingly, the microbial composition of environmental samples from the Intermodular Ventilation (IMV) Inlet of the ISS significantly overlapped with the nose microbiome while samples collected from the ISS ARED (Advanced Resistive Exercise Device) Handle Bar, Cupola Nadir Window Shade Knob, Crew Quarters Stationary Light Knob, and Handheld Microphone handles/grips showed a distribution that overlapped with the two skin samples, forehead, and forearm ($p < 0.001$, pairwise error rate for multiple testing = 0.005 [Bonferroni]). 2.2 Analysis of alpha diversity in human samples. To further investigate the effect of space travel on the human microbiome we evaluated the stability of the inverse Simpson alpha diversity index, that is a quantitative measure of the species richness and evenness in an ecosystem. This index increases with an increase in the number of species or when species are more evenly distributed in a particular ecological niche. Although our sample collections are still incomplete, alpha diversity measurements of almost-complete datasets showed some preliminary trends that are worth mentioning. For example, the gut microbiome from astronaut C showed a markedly increase in in-flight alpha diversity. A similar increase in alpha diversity was observed for forearm in-flight samples from astronauts C and H. Astronauts B and E, however, showed an opposite trend, although more time points need to be incorporated in order to have a more complete picture about the fluctuations in forearm alpha diversity. 2.3. Analysis of beta diversity in human samples. While alpha diversity measures the species diversity within each sample, beta diversity estimates the difference in species diversity between ecological niches and gives an idea of how much diversity is being shared between them. For

Task Progress:

this study we used the Yue & Clayton measure of dissimilarity index [3], that takes into account the proportion of shared and unshared species between two microbial communities.

Beta diversity analysis of stools samples from astronaut C revealed that in-flight samples were more similar to each other than to pre-flight samples ($p < 0.001$) or post-flight samples ($p = 0.006$). A similar analysis on forearm and forehead samples from astronauts C and H indicated that in-flight samples had a lower beta diversity index compared to pre-flight samples ($p = 0.007$ forearm; $p = 0.047$ forehead) but no significant differences were found between in- and post-flight samples. On the other hand, nose and tongue samples from the same two astronauts did not show any statistical difference in beta diversity among pre-, in-, and post-flight samples.

2.4 Analysis of the microbial composition of environmental samples from the ISS.

The analysis of 16S taxonomic profiles from 27 ISS surface samples and 3 samples from the water tank showed that the microbial composition of the five ISS sites surveyed resemble that of the two astronauts skin sites, forearm and forehead. Interestingly, some of the samples from the Intermodular Ventilation (IMV) Inlet and the Smoke Detector are enriched in some of the most abundant genera in the nose microbiome of the astronauts. On the other hand the water tank sample presented a less diverse microbial community compared to ISS surfaces.

3. Discussion.

In this report we presented preliminary results from the first 156 human microbiome samples and 30 ISS environmental samples received at JCVI and collected before, during, and after a mission to the ISS. Our analysis showed that the taxonomic data generated from sequencing PCR amplicons spanning the v4 region of the 16S rRNA gene from astronauts' samples were consistent with previous human microbiome studies and the HMP project. Indeed, each of the five human habitats surveyed, tongue, gut, forehead, forearm, and nose, harbored a distinctive microbiota, with forehead and forearm being the most similar and gut and tongue (dorsal) the most different. Also, in agreement with other human microbiome studies, samples collected from the same subject usually clustered together and taxonomic profiles from each sample were enriched in microbial genera typically found in the environments surveyed.

Even though most of the sequenced datasets lack several data points, it was still possible to identify some trends in those sample collections that were almost complete, mostly from astronauts C and H. Our analysis of taxonomic profiles showed that the composition of the microbial communities from subjects C and H changed during their stay in the ISS and in some cases those communities seemed to get back to their original composition 30 days after return.

In addition, analysis of alpha diversity seemed to indicate that the response of the human microbiome to the space environment is site-specific and in some cases subject-specific. Also, our study of beta diversity on subjects C and H showed that in-flight samples were more similar to each other than to pre-flight samples, supporting the hypothesis that long stays in the ISS affect the composition of the human microbiome.

Lastly, taxonomic profiling of 30 environmental samples indicated that the microbiota that inhabits different ecological niches in the ISS is most likely of human origin and mainly from human skin.

4. References

1. Structure, function and diversity of the healthy human microbiome. Nature 486, 207-14 (2012).

2. Costello, E.K. et al. Bacterial community variation in human body habitats across space and time. Science 326, 1694-7 (2009).

3. Parks, D.H. & Beiko, R.G. Measures of phylogenetic differentiation provide robust and complementary insights into microbial communities. ISME J 7, 173-83 (2013).

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