Comprehensive Microbial Diversity Characterization of the International Space Station and Analogous Closed Habitats on Earth

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Abstract

- Maintaining safety of astronauts during long-term habitation in the International Space Station (ISS) is an essential undertaking that must be attained for sustained human presence in space.
- Of great concern is the ubiquitous presence of microorganisms, which have the potential to not only affect crew health but also have adverse effects on crew systems. Increased microbial burden on ISS could lead to infection or disease outbreaks in the closed habitat.
- The threat of such an outbreak is especially hazardous due to the limited treatment and patient quarantine options.
- To date, monitoring the microbial diversity of the ISS was performed utilizing culture-based methods for detailed molecular characterization.

Methodology

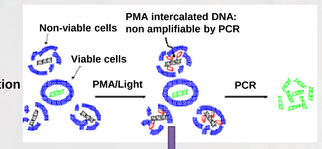
- The microbial diversity of three distinct closed habitats such as the ISS, Inflatable Lunar-Mars Habitat (ILMH) at University of North Dakota and a NASA spacecraft assembly facility (SAF) was characterized using the state-of-the-art molecular techniques.
- Particles accumulated in the vacuum cleaner bags (ISS and SAF) and the floor samples of ILMH were aseptically sampled and analyzed.
- Cultivation techniques augmented with rapid molecular techniques were used to measure total, viable, and cultivable microbial burden.
- ATP assays was used to estimate the total and viable microbial burden.
- Propidium monoazide (PMA) treatment followed by qPCR was used to estimate viable bacterial burden.
- In addition, next-generation sequencing technologies were used to elucidate the full spectrum of total and viable bacterial, archaeal, and fungal diversity.

Sample Collection Sites

ILMH

SAF

ISS



DNA Extraction

Promega.com

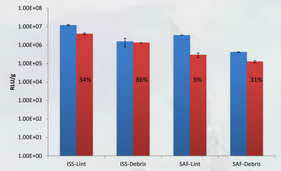
qPCR analysis

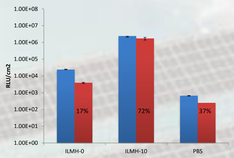
High-throughput pyrosequencing (454)

Data obtained from the 454 pyrosequencing was subjected to computational analysis to estimate the microbial diversity and community differences among the three environments .

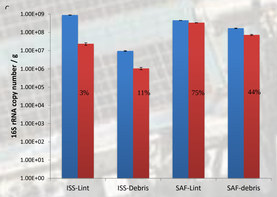
Results

Viable Microbial Burden-ATP Assay





Viable Bacterial Burden-qPCR



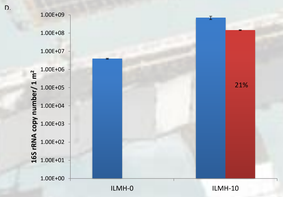
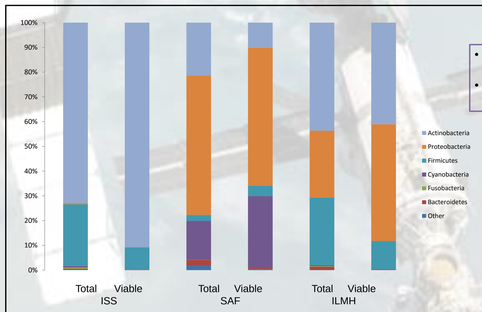
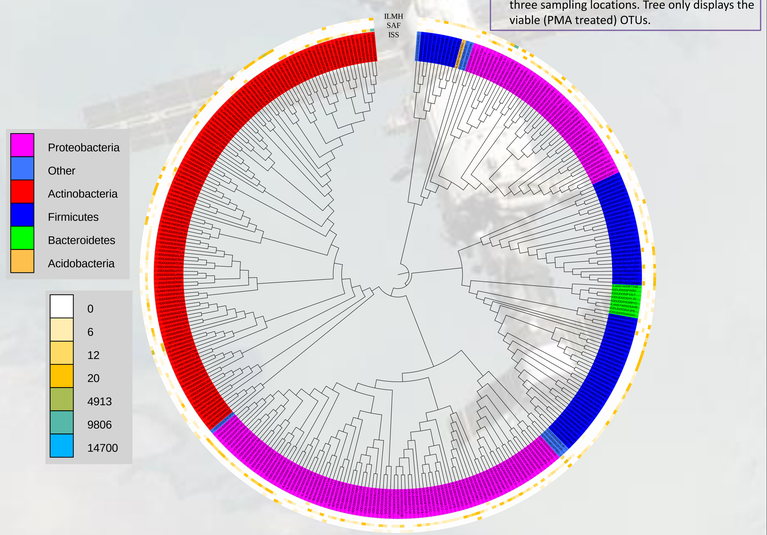


Figure A-B: Total and viable microbial burden as estimated by the ATP assay. The viable microbial population was higher in ISS when compared to SAF samples.
Figure C-D: Total and viable bacterial burden as estimated by the qPCR assay. In contrast to ATP assay, the measurement estimated by PMA-qPCR exhibited higher viable bacterial population in SAF (~45-75%) when compared to ISS (3-11%) samples. This results show that ISS debris samples might have more fungal population than the SAF samples where the high pH cleaning agents used might be detrimental for fungal survival.

The ILMH surfaces exhibited accumulation of ~10^6 microbes and ~10^8 bacterial population per m^2 after 10 days of "student astronauts" occupation. Among these population ~70% microbial and ~21% bacterial populations are viable.

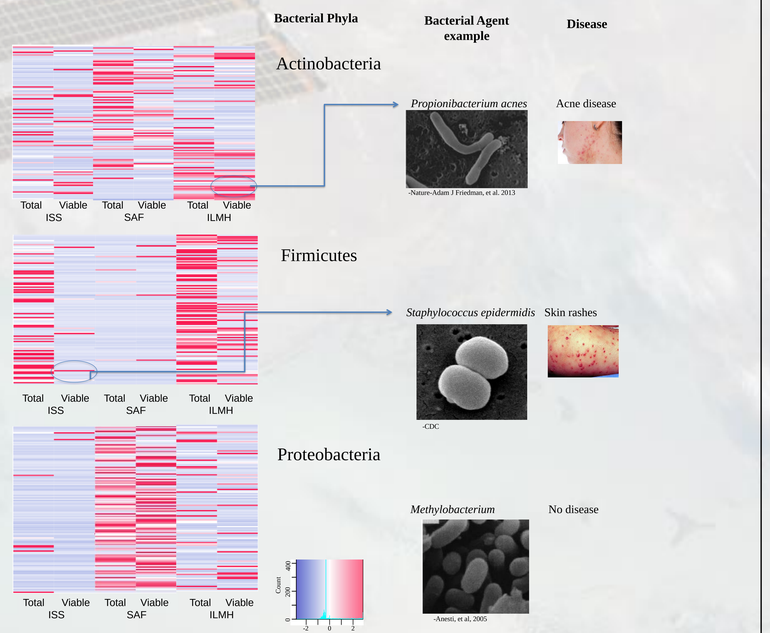


- Percent abundance of OTUs given by their Phyla. Samples treated with and without PMA.
- Similar trends with Proteobacteria abundance was observed with SAF and ILMH locations



- Phylogenetic tree showing the relationship in heatmap form of the shared OTU among the three sampling locations. Tree only displays the viable (PMA treated) OTUs.

Habitat – Specific bacterial Phyla as depicted by heat map. ISS habitat samples were enriched with Actinobacteria, SAF samples contained predominately Proteobacteria, and ILMH surface locations were abundant with Firmicute members. Since ILMH air is circulated with external air, environmental microbes such as Firmicutes were observed. Likewise, high human traffic were documented in SAF thus presence of Proteobacteria was expected. Unlike these two Earth analogues, ISS was a closed habitat and robust Actinobacterial group was evident.



Observations and Conclusions

- As estimated by ATP assay, the viable microbial burden of the debris collected from the ISS are ~1 log higher than observed in SAF cleanroom environments. SAF floors are regularly maintained with high pH cleaning agents where as such cleaning exercises are not routinely performed in ISS unless otherwise any problem arise.
- Prior to human occupation, the ILMH surfaces were kept impeccably clean due to rigorous maintenance. Subsequent 10-days ILMH occupation by "student astronauts" influenced increase in viable microbial (10^6 RLU per m^2; ATP-assay) and bacterial (10^8 *rrn* per m^2; PMA-qPCR-assay) burden in these ILMH surfaces. The source of these microbes might be attributed to the environmental control system (air exchange) and human shedding.
- Two logs of higher bacterial burden as estimated by PMA-qPCR need further research. One possible explanation for the higher bacterial signal is that by average ~10 *rrn* copies might be present in a given bacterial cell.
- The approach adapted during this study was the first ever-comprehensive molecular effort to assess the viability of microbial cells associated with space-related enclosed habitat and to correlate differential viability with phylogenetic affiliation.
- The results of this study also improved our understanding of background microbial contamination, thus facilitating the development of biosensors to monitor closed habitats like ISS and future manned missions.

Acknowledgements

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