The Viable Microbiome In Controlled Indoor Environments: Metagenome Of The Living

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Abstract

The findings of recent studies employing 16S rRNA gene sequencing and metagenomic analyses from sample-derived total DNA extracts have postulated a reciprocal dependency between indoor and human microbiomes. However, the results of this investigation demonstrate that the metagenome exclusive to viable cells differs significantly from that derived from the total DNA recovered from indoor environments. The molecular viability marker propidium monoazide (PMA) was applied to samples collected from a spacecraft assembly facility (SAF), and subsequent metagenomic sequencing experiments showed considerable differences between the resulting viable-only and total microbiomes. The composition of the viable bacterial communities associated with uncontrolled gowning areas (GA) differed significantly from that of controlled cleanroom environments, implicating selective pressure on indoor bacteriomes by more stringent facility maintenance and cleaning. Nevertheless, analyses of sequence abundance suggested that the viable microbiome was influenced by both the human microbiome and the ambient ecosystem external to the facility, which resulted in a complex community profile. Differences in sequence abundance and functional capabilities between samples suggested a decrease of oxygen-dependent organisms in the cleanroom environment. Also detected were the first viral signatures ever retrieved from a cleanroom facility: the genomes of human cyclovirus 7078A and Propionibacterium phage P14.4. The findings presented here, as well as the innovative methods that enabled their discovery, promise to have profound implications on the design and interpretation of ongoing and future indoor microbiome studies.

Methodology

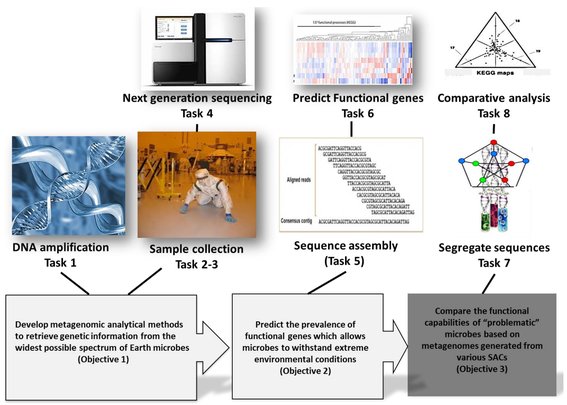
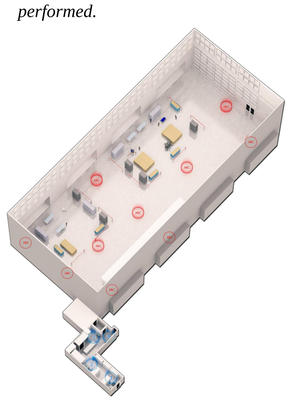


Figure 1: A comprehensive flowchart depicting the functional metagenomics project research overview.

Figure 2: A schematic representation of the spacecraft assembly facility (SAF) and gowning area (GA) and the locations where sampling was performed.



Results

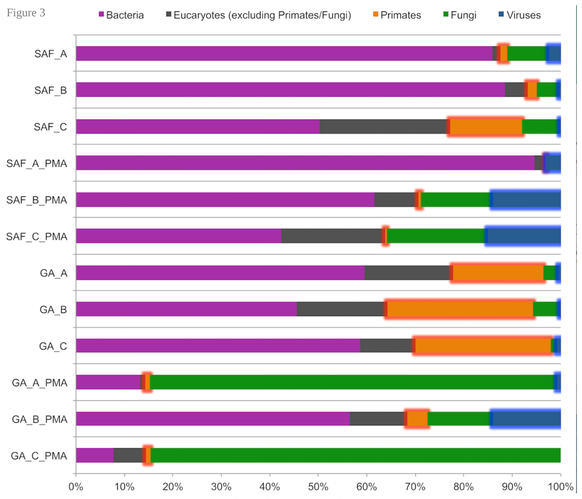


Figure 3 Proportional abundances of community subpopulations. Subpopulations showing a significant change between sample groups are highlighted with a colored frame.

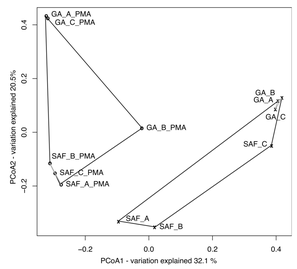


Figure 4: Ordination analyses at genus level abundances for entire community profiles.

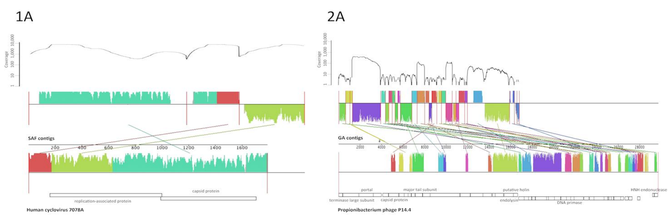


Figure 5: Whole genome alignment of assembled contigs against the viral reference genome that showed the highest similarity. Coverage distribution is indicated above alignment on log-scale. 1A: Human Cyclovirus 7078A; 2A: Propinobacterium phage p14.4

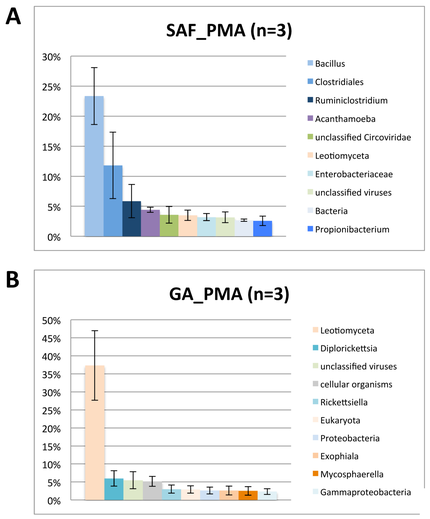
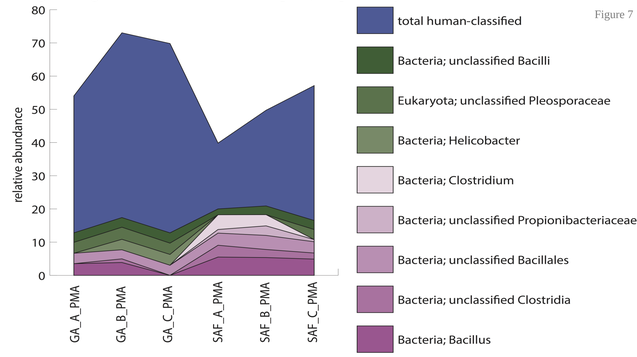


Figure 6: Rank-abundance curves of relative abundance data in SAF_PMA and GA_PMA samples. Absolute abundance of each taxon was normalized based on the total abundance of all samples considered. The top ten taxa are listed. Error-bars indicate standard deviation.

Figure 7: Relative abundance of human sequences and 8 microbial taxa whose abundances were significantly correlated with the abundance of human sequences in gowning area and cleanroom samples, respectively.



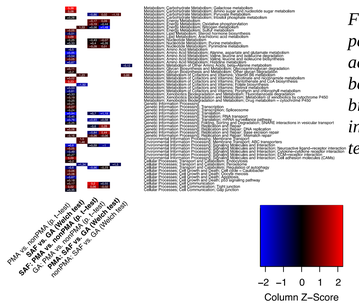


Figure 8: Heatmaps displaying 58 pathways with significant differences across sample groups differences between the viable and non-viable biome and between GA and SAF investigated using paired student's t-tests and Welch tests..

Conclusions

- Proportions of bacteria, eukaryotes and viruses vary considerably different between the viable and non-viable metagenome.
- The spacecraft assembly facility exhibits a significantly different community composition than the gowning area.
- Genome reconstruction provides evidence for the presence of human cyclovirus 7078A and Propionibacterium phage P14.4 in the cleanroom environment.
- The viable microbial community in the spacecraft assembly facility is dominated by bacteria and viruses, whereas the gowning area is rich in fungi.
- Certain viable microbial taxa showed a significant correlation to human abundance
- The cleanroom harbors more facultative and obligate anaerobes and spore-forming taxa.

Acknowledgement

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