XXX

New Perspective on Cleanrooms and Human Pathogens.

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Abstract

Strict planetary protection practices are implemented during spacecraft assembly to prevent inadvertent transfer of earth microorganisms to other planetary bodies. Spacecraft assembly clean rooms undergo strict cleaning and decontamination procedures, which lead to reduction in total microbial bioburdan. We wanted to evaluate if these practices selectively favors survival and growth of hardy microrganisms, such as pathogens. Three geographically distinct clean rooms were sampled during the assembly of three NASA spacecraft: Dawn, Phoenix, and Mars Science Laboratory mission. During Phoenix mission, sample sets were collected from clean room at three time points: before arrival of the Phoenix spacecraft, during the assembly and testing of the Phoenix spacecraft, and after removal of the spacecraft from the PHSF facility.

All samples were subjected to whole genome shotgun metagenome sequencing on an Illumina HiSeq 2500 platform. We screened for pathogens and other virulence factors, which determine pathogenicity. Potential pathogens and their corresponding virulence factors were present in all the samples. Though the relative abundance of pathogens was lowest during the Phoenix assembly, virulence factors increased from before to during to after assembly, potentially offering a survival advantage. Decreased microbial and pathogenic diversity indicates that decontamination and preventative measures were effective and well implemented. This is the first metagenome study describing presence of pathogens in controlled enclose environments and their corresponding virulence factors. The results of this study should be considered for enclosed environments, humans on the International space station and in planning for future human missions to Mars.

Methodology

Sample sets were collected from the Kennedy Space Center's Payload Hazardous Servicing Facility (KSC-PHSF) cleanroom floor surfaces at three time points: before arrival of the Phoenix spacecraft (10 samples; PHX-B), during the assembly and testing of the Phoenix spacecraft (8 samples; PHX-D), and after removal of the spacecraft from the KSC-PHSF facility (10 samples; PHX-A), Lockheed Martin Aeronautics' Multiple Testing Facility (10 samples; LMA-MTF) cleanroom floor during the DAWN spacecraft assembly and from the Ground Support Equipment (GSE) at Jet Propulsion Laboratory's spacecraft assembly facility (2 samples; JPL-SAF) during the Mars Science Laboratory (MSL) spacecraft assembly.

DNA was extracted from each concentrated sample using bead beating and an automated DNA extraction instrument (Autolyser A-2 DNA, Axcyte Genomics, Menlo Park, CA) and archived at -80 ° C until further use.

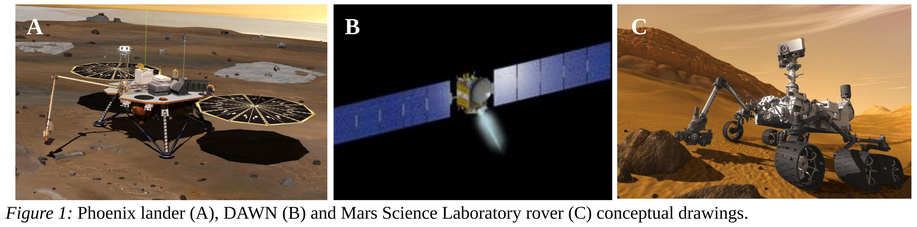

Figure 1: Phoenix lander (A), DAWN (B) and Mars Science Laboratory rover (C) conceptual drawings.

Results

	PHX-B	PHX-D	PHX-A	DAWN	MSL
Sum	24	2867	5458	16	501
Sum norm.	2	241	334	96	15
Virulence diversity	14	48	41	9	6
Virulence diversity norm.	1	4	2	54	0.2
Pathogenic diversity*	3	6	11	3	2

Table 1: **Virulence factors with their corresponding pathogens.**
norm: normalized to counts per million reads
* number of pathogens with ≥ 1 corresponding virulence factors

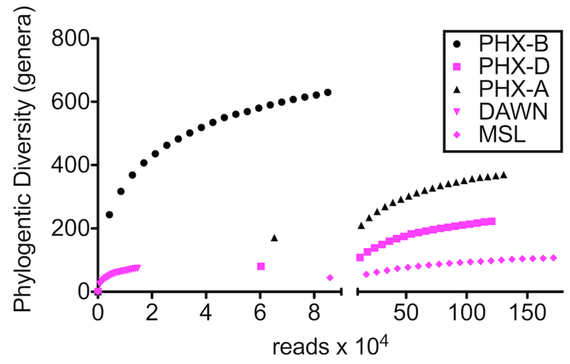

Figure 2: Rarefaction curves of samples taken during assembly (PHX-D, DAWN, MSL) show less genera at the same sample size compared to samples taken before (PHX-B) and after (PHX-A) assembly.

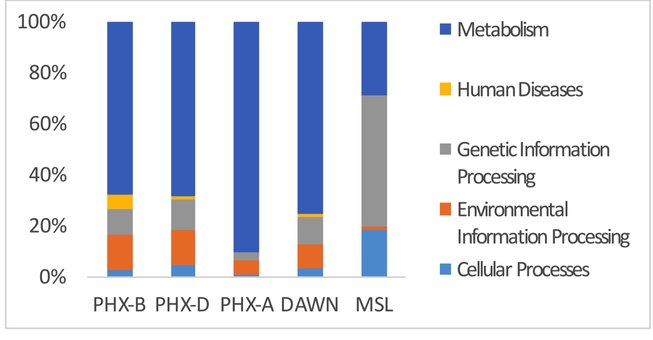

Figure 3: **Metabolic genes cover majority of functional classifications.** KEGG Pathway analysis of PHX-B, PHX-D, and PHX-A, DAWN and MSL. During assembly of PHX-D and DAWN, we see that a bigger fraction of all classified sequences have been assigned to metabolism. .

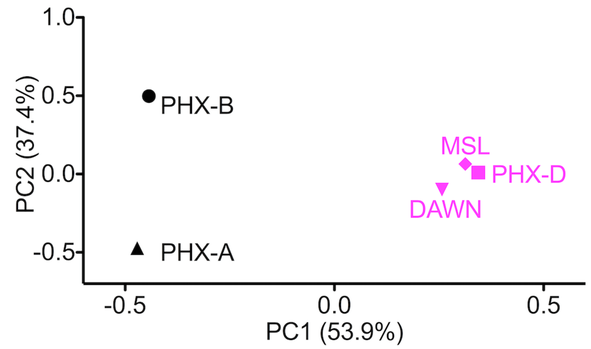

Figure 4: Principle coordinates analysis on genus taxonomic level based on a Bray-Curtis dissimilarity matrix. Samples taken during spacecraft assembly MSL, PHX-D and DAWN showed a similar community profile although these facilities were geographically distant.

Conclusions

- This is the first functional metagenomics study describing presence of pathogens and their corresponding virulence factors in cleanroom environments.
- Decreased phylogenetic and pathogenic diversity indicates that decontamination and preventative measures were effective and well implemented, but pathogen abundance still increased over time.
- Four potential pathogens, *Acinetobacter baumannii*, *Acinetobacter lwoffii*, *Escherichia coli* and *Legionella pneumophila*, and their corresponding virulence factors were present in all cleanroom samples
- Though the relative abundance of pathogens was lowest during the Phoenix assembly, potential virulence factors were higher during assembly compared to before and after assembly

Acknowledgement

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