Metagenomics Approach to Predict Functional Capabilities of Microbes in Clean Room Facilities

Alexander Mahnert[1,2], Parag Vaishampayan[2], Kasthuri Venkateswaran[2], Gabriele Berg[1], and Matt Christensen[2]

[1]*Institute of Environmental Biotechnology, Graz University of Technology, Graz, Austria*
[2]*Biotechnology and Planetary Protection Group, Jet Propulsion Laboratory, California Institute of Technology, Pasadena, CA*

Background

Metagenomics approach provides a comprehensive microbial census as well as the functional potential of a microbial community surviving in a given habitat. We will adopt well-developed Metagenomics approaches to Predict Functional Capabilities (MPFC) of the microbial community present on spacecraft and associated surfaces. The MPFC team will utilize next generation sequencing technologies and advanced bioinformatics capabilities that have been developed and implemented by the Joint Genome Institute (JGI), a co-investigator institution.

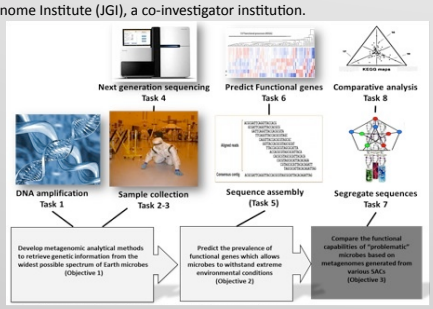


Figure 1: A comprehensive flow chart depicting the Functional Metagenomics project research overview.

Introduction and Study Concept

Clean rooms are maintained to their specific certification and are controlled indoor environments with increasing importance for modern industrial manufacturing processes. Despite stringent cleaning and maintenance clean rooms are not sterile and many microbes withstand those extreme oligotrophic conditions (La Duc *et al*., 2007; Moissl *et al.,* 2007).

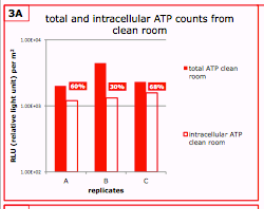
Modern molecular methods are capable of measuring comprehensive microbial burden/ diversity about any given environment in contrast to cultivation based methods. However, an often neglected fact of molecular approaches is their disability to differentiate viable portions of microbes from dead cells (Vaishampayan *et al.,* 2013). In addition to ATP (adenosine triphosphate) assay, which was shown to assess viable and active fractions of microbes (Venkateswaran *et al.,* 2003), the DNA intercalating dye PMA (propidium monoazide) was also used during this study.

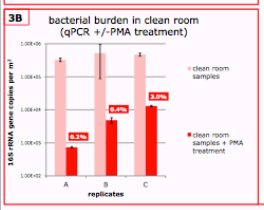
The utilization of PMA to mask DNA from dead cells is an elegant method to distinguish intact and viable cells from dead microorganisms with compromised cell walls on molecular level (Nocker *et al.,* 2007; Rawsthorne *et al.,* 2009; Vaishampayan *et al.,* 2013).

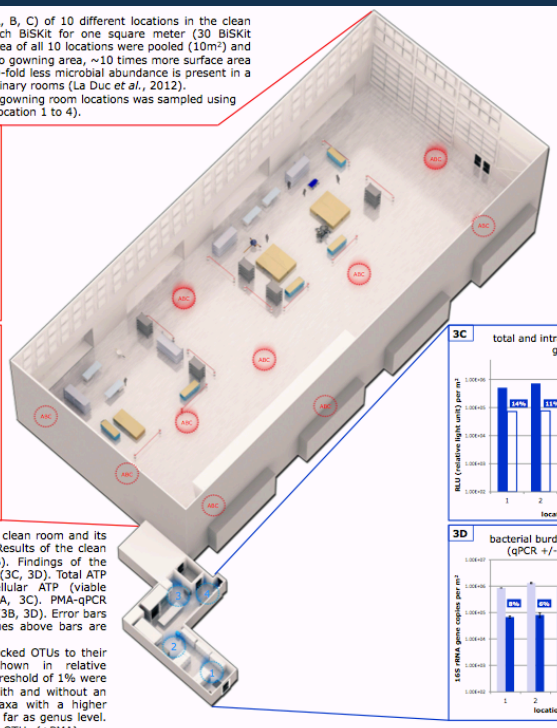
Here we quantified viable microbial bioburden of a spacecraft assembly clean room (Class 100K) at NASA Jet Propulsion Laboratory and its adjacent servicing facility (gowning room). Furthermore we give an insight into microbial diversity of both rooms. Floor samples were collected using Biological Sampling Kit (BiSKit) from several locations and appropriate samples were pooled before subjecting to molecular analyses. The ATP-assay was used to determine relative differences between both sampled rooms concerning their active microbial fractions. qPCR was applied to quantify variations of PMA treated and untreated metagenomic DNA portions.

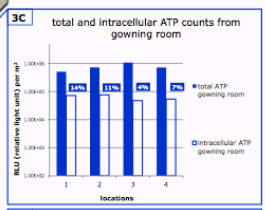
Results

Sampling details: Three adjacent areas (A, B, C) of 10 different locations in the clean room (red circles) were sampled using each BiSKit for one square meter (30 BiSKit samplers for 30m² in total). Each adjacent area of all 10 locations were pooled (10m²) and analyzed for molecular analyses. Compared to gowning area, ~10 times more surface area was collected, since it was reported that ~10-fold less microbial abundance is present in a Class 100K room compared to uncertified ordinary rooms (La Duc *et al.*, 2012).
One square meter surface area of 4 different gowning room locations was sampled using BiSKit samplers (blue circles; designated as location 1 to 4).









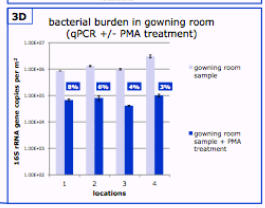
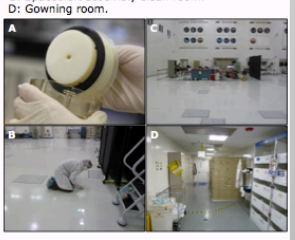
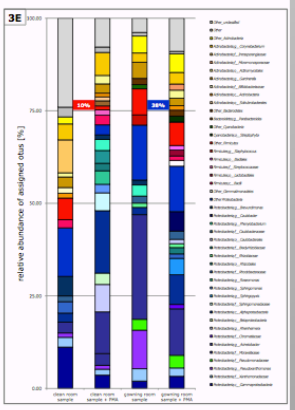


Figure 3: A 3D-rendered-model of a typical clean room and its adjacent servicing facility (gowning room). Results of the clean room are displayed in red colors (3A, 3B). Findings of the gowning room are highlighted in blue colors (3C, 3D). Total ATP (dead and viable microbes) and intracellular ATP (viable microbes) was determined in duplicate (3A, 3C). PMA-qPCR measurements were carried out in triplicate (3B, 3D). Error bars in diagrams show standard deviations. Values above bars are percentage of viable microbial population.
3E) Microbial diversity and assignment of picked OTUs to their phylogenetic groups. Proportions are shown in relative abundance [%]. Taxa which lay beneath a threshold of 1% were grouped in the artificial category "Other" with and without an indication to their representing phylum. Taxa with a higher relative abundance than 1% are resolved as far as genus level. Values between bars are percentage of viable OTUs (+PMA).

Figure 2: Sampling characteristics.
A: Biological sampling kit (BiSKit).
B: Active collection of clean room floor sample.
C: Spacecraft assembly clean room.
D: Gowning room.





Conclusion and Future Work

- Viable microbial population (measured by ATP; ~10^3 cells/m²) was less in the spacecraft assembly facility clean room than the adjoining uncertified gowning facility (~10^4 cells/m²).
- Even though percentage of viable cells was recorded high in the clean room locations (~50%) when compared to the gowning area (~4 to 14%), the abundance of viable cells was one-log less in the clean room surface area. This reiterates the fact that the stringent maintenance of JPL spacecraft assembly clean room floors might have removed excessive number of microbial cells but some selective microbial populations were able to survive under these clean conditions.
- The qPCR results of PMA untreated samples showed one-log (gowning area) or two-logs (clean room locations) higher bacterial population than total ATP assay measured microbial abundance. This might be due to the fact that the DNA from the dead cells might have persisted even after cleaning whereas ATP might have degenerated due to alkaline nature of the cleaning reagents used.
- The qPCR results of PMA-treated samples (viable microbes) exhibited similar abundance of microbial population (~10^4 cells/m²) as measured by ATP assay in gowning area.
- The microbial abundance as estimated by the PMA-qPCR was ~10^2 to 10^4 bacterial cells/m2 and by ATP assay it was ~10^3 microbial cells/m² in clean room locations.
- As a next step 16S rRNA-gene targeted Illumina MiSeq based next-generation sequencing will determine the persistent microbial species in the clean -and gowning room. A preliminary view on microbial diversity is given in Figure 3E. In addition, a metagenomic approach targeting various genes is planned to reveal the presence of active functional microbial species.

Acknowledgements:
We would like to thank Jessica Cisneros (JPL), Anna Auerbach, Alexander J. Probst, Christine Moissl-Eichinger (University of Regensburg) and Thomas Dohr for their professional help and input. Part of this work was carried out during a JPL Visiting Student Research Program at NASA Jet Propulsion Laboratory, California Institute of Technology and was supported by the KUWI scholarship of the Graz University of Technology.

REFERENCES:
1. La Duc MT, Dekas A, Osman S, Moissl C, Newcombe D and Venkateswaran K. (2007). *Appl Environ Microbiol* **73**: 2600-26112.
2. La Duc, M.T., Vaishampayan, P., Nilsson, H.R., Torok, T., and Venkateswaran, K. (2012) Appl Environ Microbiol, **78**:5912-5922.3.
3. Moissl C, Osman S, La Duc MT, Dekas A, Brodie E, DeSantis T and Venkateswaran K. (2007). FEMS Microbiol Ecol **61**:509-5214.
4. Nocker A, Sossa-Fernandez P, Burr MD and Camper AK. (2007). Appl Environ Microbiol **73**:5111-51175.
5. Rawsthorne H, Dock CN and Jaykus L. (2009). Appl Environ Microbiol **75**:2936-29396.
6. Vaishampayan P, Probst AJ, La Duc MT, Bargoma E, Benardini JN, Andersen GL and Venkateswaran K. (2013). ISME J **7**:312-324 7.
7. Venkateswaran K, Hattori N, La Duc MT and Kern R. (2003). J Microbiol Meth **52**:367-377.

Institute of Environmental Biotechnology
Graz University of Technology • Austria
email: a.mahnert@tugraz.at

Jet Propulsion Laboratory
California Institute of Technology