Title:
Culture-Independent Microbial Air Profiling using a Spaceflight-Compatible Nanopore Sequencing Method

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Abstract:
Microbial monitoring of spacecraft air is critical toward assessing the efficacy of microbial controls within the environmental control and life support systems to protect both the crew and the vehicle environment. Additionally, understanding of the atmospheric microbial profiles will be essential toward Mars-forward planetary protection planning. Currently, onboard the International Space Station (ISS), the air is monitored on a quarterly basis using an impaction air sampler. With this method, bacterial and fungal cells and spores are pulled onto plates containing growth medium. Following five days of ambient incubation onboard the ISS, the crew compares the growth to density charts and provides the approximations to the ground. Upon return of the culture plates to the Johnson Space Center's Microbiology Laboratory, the isolates present are identified for crew health risk assessments. As NASA moves beyond the low-Earth orbit of ISS, sample return will be impractical. As such, a near real-time monitoring capability for the assessment of the spacecraft atmosphere is necessary.

Significant strides have been made in recent years to utilize a molecular-based method for microbial profiling of ISS surfaces. The developed method is not dependent on microbial culture, thus removing the associated risk to the crew from high microbial levels, the bias toward detecting only the culturable organisms, and eliminates the need for sample return. The work described here details the evaluation of three different air sampling platforms whose product is amenable to downstream molecular processing and nanopore sequencing. The three samplers were compared in terms of mass and power requirements, ease-of-use, and the resulting data. For the two highest ranking samplers, a basic concept of operations was developed to transfer the sample into the already established preparation and sequencing process. Using these concepts of operations, an in-depth comparison of the molecular data generated was compared to the historical culture-based method. Data from both methods detailed similar microbial profiles, while the molecular method detailed microbial identifications that could not be identified through the culture-based method. The developed method will enable the generation of near real-time microbial profiles of the spacecraft atmosphere.