

Abstract

This study was performed embedded in the overall goal to establish a method evaluating a ratio between dead and living organisms due to their environment in the European Space Agency's cleanrooms and space-travels. Since conventional analysis of microbes doesn't provide any data about their life status, this method will support delivering more information about effectiveness of microbial removing or bacterial transportation in outer space. To extract such information, the photo-reactive functionality of propidium iodide (PI) of the propidium monoazide (PMA) treatment method was used to separate nonliving from living cells by preventing amplification of DNA from nonliving organisms. Considering the importance of time consumption in spacecraft assembly facilities, this method should take less than eight hours to process. For comparable evaluation and initial assessments, the focus is on microorganisms such as Gram-positive bacteria *Solibacillus* or *Microbacterium oleivorans*, but also ubiquitous microbes from different environments. The microorganisms were exposed to various extreme conditions, such as radiation, desiccation or freezing at the German Aerospace Center (DLR) and then treated with the new method, which defines the ratio between viable/dead cells. Due to the planetary protection guidelines, defined by the Committee on Space Research (COSPAR), this method is meant to get information about the impact of chemical and physical burdens to microbial organisms. All qPCR results from PMA treated communities, indicated a reduction of number of amplicons, which is proportional to the number of living cells, in the view of blocking amplification of DNA from nonliving cells. However, the used amount of PMA seems too low to block all of non-intact cells. Compared to desiccation tests, freezing exposures showed the biggest impact on reducing living cells, followed closely by radiation. The final steps were to sequence all treated samples using the next generation sequencing method. Analysis of the obtained amplicon data showed a lower biodiversity of samples treated with PMA compared to untreated ones.

Keywords: genomic extraction, classification, bioinformatic, PMA treatment, stress tests.