Applications of Bioluminescence-based Assay in Monitoring Microbial Burden

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Guidelines for space missions to other planetary bodies, as well as for extraterrestrial sample return, stress the importance of avoiding contamination of hardware components with terrestrial organisms, their remains, and organic matter in general. Viable organisms are of particular concern, but methods for their detection and enumeration are fraught with difficulties, notably the fact that different species require different media for growth, so in practice all can never be detected as colony forming units. A firefly luciferase bioluminescence assay that differentiates free extra-cellular (dead cells, etc.) from intra-cellular (viable microbes) ATP was used to determine the microbial cleanliness of NASA spacecraft assembly rooms. The viable microbial population as evaluated by ATP was one to 3-logs higher than that indicated by aerobic plate counts. Six major clusters were identified based on ATP content and purified isolates representing 5 of these clusters except viable but non-cultivable microbes were identified by 16S rDNA sequence analysis and their intracellular ATP concentrations were measured. The 16S rDNA sequences retrieved from these samples that had no cultivable microbes but measurable ATP showed high similarities with the non-cultivable species such as members of oligotrophic, thermophile, anaerobic, and Ultermicrobacterium sp. (cell volume 0.3 µm³) etc. In addition, since the AMP level is much higher than the ATP level in spores, AMP may be a better biomarker for spore detection. In a bioluminescence assay developed by Kikkoman Corp., AMP is converted to ATP using pyruvate orthophosphate dikinase, and ATP is subsequently detected by luciferase. We measured the AMP content of several spores of Bacillus species using AMP- bioluminescence assay and optimized conditions suitable for rapid spore detection. This AMP based spore assay is rapid and sensitive. It may have wide applications in homeland security, defense against bio-warfare agents, environmental monitoring, as well as in the food and pharmaceutical industries.