Fluorescence-Activated Cell Sorting of Live Versus Dead Bacterial Cells and Spores

Commercial applications include hospital operating room cleanliness validation assays, pharmaceutical development, and semiconductor development.

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This innovation is a coupled fluorescence-activated cell sorting (FACS) and fluorescent staining technology for purifying (removing cells from sampling matrices), separating (based on size, density, morphology, and live versus dead), and concentrating cells (spores, prokaryotic, eukaryotic) from an environmental sample.

Currently, the state of the art is limited to the sorting of larger eukaryotic cells (e.g., yeast, mammalian). Over the past decade, cell sorting technologies have evolved significantly and sensitivity levels have increased remarkably, rendering bacterial cell sorting a feasible concept. In parallel, optimized protocols for broad-spectrum fluorescence staining of bacterial cells and spores have been established, most of which are based on nucleic acid-intercalating dyes.

Small DNA-intercalating dyes, such as SYTO-9, permeate the intact membrane of living, viable cells and spores and upon excitation with white light, emit a detectable signal such as the green spectra emitted by DNA-bound SYTO-9. A larger DNA-intercalating dye such as 7-amino actinomycin (7-AAD), which is unable to permeate the membranes of healthy, viable cells and spores and thus only able to access the DNA of dead or dying cells and spores through compromised membranes, is also applied to the sample. This larger dye is engineered to fluoresce red spectra upon excitation. Ergo, the membranes of healthy, viable bacterial cells and spores preclude the infiltration of the larger red dyes (which have a greater affinity for DNA than the smaller green dyes) and as a result, their DNA fluoresces green. The DNA of dead or dying cells and spores fluoresces red as a result of the high-affinity binding and of the larger red dyes. This motif makes possible the ability to sort and segregate live from dead bacterial cells and spores via fluorescence staining.

This technology directly contributes to NASA missions as it focuses on the separation, purification, and concentration of cells or spores from a given spacecraft or associated facility sample. Coupling live/dead fluorescence dyes and flow cytometry enhances the resolving power of any attempt at predicting the microbial genetic that actually poses a forward contamination threat. The capability to provide an account of the living organisms present on spacecraft surfaces, to the exclusion of the expired population, will facilitate much more accurate predictive risk assessments of forward contamination on missions with challenging planetary protection issues. A specific account of only the living microbial population will also allow for immediate feedback to a project as to the success of cleaning, microbial reduction, and general housekeeping processes.

This work was done by James N. Benardini, Myron T. La Duc, Rochelle Diamond, and Josh Verceles of Caltech for NASA’s Jet Propulsion Laboratory. Further information is contained in a TSP (see page 1), NPO-48176.