

Use of the spore photoproduct lyase (*spkB*) gene as a marker for the detection and enumeration of spore-forming microorganisms

Tammy Ma¹, Heather Maughan², Myron La Duc¹, Roger Kern¹, Wayne Nicholson² and Kasthuri Venkateswaran¹

¹Biotechnology and Planetary Protection Group, NASA Jet Propulsion Laboratory

²Department of Veterinary Science and Microbiology, University of Arizona

Spore-forming microorganisms pose one of the largest threats to environments that are required to be sterile. Unique to spore-forming bacteria is the *spkB* gene, which encodes the enzyme Spore Photoproduct Lyase. By quantitatively detecting the presence of *spkB* in a swabbed surface sample, it is possible to evaluate the burden of spore-forming organisms, thus the relative cleanliness of the surface. Twenty-eight *Bacillus* species were procured from various sources, and their DNA was extracted by both manual and automated methods. The 16S (*rrn*) and *spkB* genes were then PCR amplified, and species showing positive *spkB* gene amplification were sequenced. Alignment of the *spkB* sequences enabled us to identify highly conserved domains for the design of semi-degenerate "universal" *Bacillus spkB* primers for PCR amplification of unknown samples.

The *spkB* gene nucleotide sequence is highly heterogeneous and ~70% nucleotide sequence similarity was observed among various species of *Bacillus*, as well as between inter-genus spore-forming bacteria. Such heterogeneity of gene sequence has been exploited to design effective probe-primer sets specific for a given problematic species. For example, a specific TaqMan *spkB* probe-primer set was synthesized that allowed us to perform quantitative real-time PCR to detect *B. subtilis* from environmental surface samples. Surfaces contaminated with as few as 10³ CFU were effectively detected using this TaqMan system. We are currently designing sampling methods to increase the sensitivity of this viable methodology for rapid and quantitative detection of spore-forming microorganisms. The use of such a system for the detection of biowarfare agents, such as *B. anthracis*, is currently being explored.