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

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Spore-forming microorganisms pose one of the largest problems in maintaining ultra-clean environments, such as spacecraft and their assembly facilities. Unique to spore-forming bacteria is the splB gene, which encodes the Spore Photoproduct Lyase enzyme. It is possible to evaluate the burden of spore-forming organisms in a given sample by quantitatively detecting the presence of splB. Thirty-five Bacillus strains were procured from various sources, and their DNA was extracted by both manual and automated methods. The 16S (rrn) and splB genes were PCR amplified, and species showing positive splB gene amplification were sequenced. Alignment of the splB sequences enabled the identification of highly conserved domains for the design of semi-degenerate "universal" Bacillus splB primers for PCR amplification of unknown samples.

The splB 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 splB 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 the 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.