Use of *gyrB* to re-evaluate the phylogenetic characterization and nomenclature of the *Bacillus anthracis-cereus-thuringiensis* clade

MYRON T. LA DUC[1], ROGER KERN[1], MASATAKA SATOMI[2], SHOICHI YAMADA[3], AND **KASTHURI VENKATESWARAN[1]**

[1]Biotechnology and Planetary Protection Group, NASA Jet Propulsion Laboratory
California Institute of Technology, Pasadena, CA 91109
[2]National Research Institute of Fisheries Science, Food Processing Division,
Yokohama-City, Kanagawa 236-8648
[3]Nippon Suisan Kaisha, Hachioji City, Tokyo 192, Japan

The genus *Bacillus* is comprised of several microbes of notable importance to humans. *B. thuringiensis*, a well characterized insecticidal toxin producing bacterium, *B. cereus*, a model Gram-positive organism capable of causing human illness, and *B. anthracis*, the causative agent of the human disease anthrax, all group together within a very tight clade phylogenetically, when characterized by 16S rDNA sequence analysis. It is imperative to devise a system capable of accurately differentiating these closely related, yet phenotypically distinct species of *Bacillus*. It is of utmost medical importance to distinguish human disease causing microbes from their closely related, non-pathogenic relatives. Although the rRNA gene (*rrn*) has been used for decades for phylotyping, the use of this gene has attracted criticism because of its inability to decipher very closely related microbes. We propose here the sequence analysis and phylotyping of a more highly evolving gene, *gyrB*, located approximately 5-kb from the chromosomal origin of replication (*oriC*). Like other housekeeping genes, *gyrB* encodes a single copied protein, most mutations within which prove lethal. We present here the findings of a study in which the *gyrB* gene was used to phylogenetically characterize this closely-knit clade of *Bacillus*. The results showed a high degree of variation (9 to 13% of 1.2-kb) in *gyrB* amongst type strains, whereas *rrn* gene sequence dissimilarity varied by only 2 to 4 base pairs of the total 1.5-kb sequence. In addition to the type strain, both *gyrB* and *rrn* genes of 18 serotypes of *B. cereus*, 4 serotypes of *B. thuringiensis*, and 2 sequences of *B. anthracis* were phylogenetically analyzed. The *gyrB* gene proved to be much more highly differential than the *rrn* gene, yet conserved enough to design a universal PCR primer set, which led to major changes in the phylogenetic arrangement and nomenclature associated with this clade. DNA-DNA reassociation values of these *Bacillus* species supported *gyrB*-based phylogeny rather than *rrn*-based analysis. Based on the findings of this study, we propose a more accurate restructuring and naming of the bacterial strains within this clade.