Species Differentiation of the *Bacillus Cereus* Group using the *pycA* Gene Sequence and an Assessment of its Operational Impact for Pharmaceutical Manufacturers

Joseph Danner, Sunhee Hong, Bindhu Varghese, and Christine E. Farrance - Charles River Laboratories/Accugenix, Newark, DE

**ABSTRACT**

The *Bacillus cereus* group (BCG) is of high importance in the scientific, industrial, and pharmaceutical communities as members of the group are often found as contaminants in food products and manufacturing facilities. In late 2017, the description of an additional nine novel species was published. As a result, the BCG now includes 17 validly published species. Phylogenetic analysis based on the 16S rDNA sequence has traditionally been used to differentiate between organisms in this group. However, the publication of these additional new BCG species brings a new level of complexity as the 16S sequences for many type species in the group are now identical. Therefore, sequences within the BCG are too closely related to be accurately differentiated by 16S.

Since organisms in the Bacillus cereus group can trigger different levels of concern, rapid and accurate classification and taxonomic separation of the BCG species are critical for investigations in industrial settings. We have evaluated numerous protein-coding gene sequences that have been reported to be phylogenetically discriminatory and concluded that the *pycA* gene is an ideal target for distinguishing all the BCG species under consideration. This method was tested with over 300 diverse samples and a 92% sensitivity rate was obtained.

**INTRODUCTION**

The members of the *Bacillus cereus* group (BCG) of organisms are very closely related and currently include *Bacillus cereus*, *Bacillus thuringiensis*, *Bacillus weihenstephanensis*, *Bacillus mycoides*, *Bacillus pseudomycoides*, *Bacillus dracunculorum*, and *Bacillus cytotoxicus*. Late in 2017, we discovered a research article which included the publication of nine new valid species in the BCG (Table 1).

The BCG is of great importance as its members are often found as contaminants in many manufacturing facilities and food products. In addition to this, for many years, isolates identifying as *B. cereus* have been one of the most frequently submitted organisms to our facilities. Phylogenetic analysis based on the 16S rDNA gene sequence has traditionally been used to differentiate between organisms in this group. However, the publication of the additional new BCG species brings a new level of complexity (Figure 1). There are many type species in the group with identical 16S rDNA sequences, and overall the species are too closely related to be accurately differentiated. Therefore, 16S rDNA gene sequencing, used by our Accugenix-ID/BacSeq service, is no longer adequate to differentiate members of this group. Since organisms in the *Bacillus cereus* group are known to trigger different levels of concern and corrective action for manufacturers, the classification and taxonomic separation of the BCG species is critical for investigations and environmental monitoring programs.

Since organisms in the *Bacillus cereus* group are known to trigger different levels of concerns and corrective action for manufacturers, the classification and taxonomic separation of the BCG species is critical for investigations and environmental monitoring programs. In this study, we have carried out extensive research to determine alternative gene targets to the 16S rRNA gene, that would be able to discriminate between the BCG species.

We have evaluated numerous protein-coding gene sequences that have been reported to be phylogenetically discriminatory and found that the *pycA* gene is an ideal target for distinguishing all the BCG species under consideration.

**OPERATIONAL IMPACT**

In order to evaluate the operational impact of the newly developed pycA assay, we gathered more than 300 diverse samples that would potentially fail to identify to a species with the addition of the nine new BCG species. These samples were sequenced using the partial pycA gene fragment and neighbor joining (NJ) trees were constructed to infer phylogenetic relationships. As seen in the example representative, MALDI-TOF and 16S rRNA gene sequencing methods (Figures 2A and 2B, respectively) were unable to provide a conclusive species-level identification. However, as you can see below (Figure 3C), if we use the pycA gene sequence as an alternative target, we can obtain better resolution. By analyzing over 300 samples, we were able to achieve a 92% species-level ID rate (Figure 4). Despite this high success rate, there were still about 8% of the samples that failed to show phylogenetic consistency for the *Bacillus cereus* group.

**REFERENCES**