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# Discrimination between *B. cereus* and *B. thurengenesis* using 16S rDNA sequencing

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## ABSTRACT

**This paper mainly emphasizes on the methods for the identification and discrimination of closely related species. Two isolates resembling Bacillus thuringenesis were isolated from soil and dead insects and analyzed by traditional biochemical methods. Biochemical results for this isolate were similar to that of Bacillus thuringenesis. For further confirmation a set of synthetic oligonucleotide homologous to broadly conserved sequence was used for in-vitro amplification via polymerase chain reaction followed by direct sequencing. The 16S rDNA sequencing after Blast analysis confirmed the bacterial isolate as Bacillus cereus, which causes food born disease syndrome associated with enterotoxin, whereas Bacillus thuringenesis is an insect pathogen. The 16S and 23S rRNA currently considered as the most useful molecules for the determination of prokaryotic phylogeny. Analysis of these rRNA sequences has resulted in tremendous expansion of our knowledge of prokaryotic diversity and has demonstrated the limitations of the existing prokaryotic taxonomy which is based primarily on the analysis of phenotypic traits. Discrimination between B. cereus and Bacillus thuringenesis is a challenging debate to which this paper makes contribution. Thus it is difficult task to discriminate between closely related species like Bacillus thuringenesis from Bacillus cereus by any traditional methods and the fact that they have grouped together in our analysis and other recent studies is not surprising.**

**Key words :** *Bacillus cereus, B. thuringenesis,* 16S rDNA sequencing and bacterial identification

## INTRODUCTION

Spore forming micro-organisms are widely distributed in nature due to resistance in their endo-spores to various stresses and their long term survival under unfavorable conditions. Therefore, most aerobe spore-formers can be isolated form a wide variety of sources including foods. Bacillus species are important, as food spoilage organism and can be isolated from fruits, vegetable products, nuts, cereals, milk and dairy products, meat, dried foods and spices. The current classification of Bacillus isolated from dead insects is based on endosperm and sporangium morphology and further subdivision to species level on the basis of biochemical tests. Differentiation between *Bacillus* can be difficult and a large number of phenotypic tests are used to distinguish between them, although sometimes only a single feature separates species. Several *B. cereus* isolates are present in food and involved in food poisoning so these isolates needs to be properly identified by using specific and sensitive methods. As the traditional methods are insufficient to discriminate *Bacillus thuringenesis* and *B. cereus* like microbes many recent studies grouped them together (Carlson *et al.*, 1994 and Daffonchio *et al.*, 2000).

## MATERIALS AND METHODS

Bacterial Isolates: Bacillus isolate PDKV-II included in this study was collected from soil and dead insects from fields of PDKV campus. Isolated bacteria were grown on nutrient broth and used for analysis.

### *Morphological cultural and biochemical characterization of isolates or conventional identification of isolates:*

For identification of *Bacillus* spp. in our lab morphological characteristics such as shape, size, endspore production etc. were observed. Cultural characteristics were also studied along with growth in broth. Slides were observed under phase contrast microscope. Bacterial isolates and were also identified by conventional biochemical methods comprising of IMVIC test (Indole, Methyl Red, Voges Proskauer and Citrate Test), sugar fermentation eg. Glucose, lactose, maintol, arabinose and sucrose with acid and gas production and enzymes like catalase, oxidases, urease, gelatinase, amylase, caseinase, lecthinase, deaminase, cellulase, lipse, β-galactosidase, nitrate-reductase were studied (Deshpande *et al.*,).

### *Genotypic identification:*

Pure cultures of Bacilli were used for DNA extraction using the Prep-Man$^{Tm}$. DNA extraction protocol was provided in the Microseq Manual. A 500bp 16S ribosomal DNA (rDNA) fragment was amplified from 5'

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