

Certificate of Analysis for NR-12151

Bacillus cereus, Strain Tor 16585

Catalog No. NR-12151

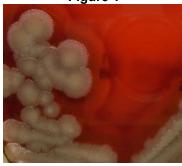
Product Description: Bacillus cereus (B. cereus), strain Tor 16585 was isolated from left arm tissue of a patient from Long Island, New York with an open fracture on August 12, 2005.

Lot¹: 58893068 Manufacturing Date: 29OCT2009

TEST	SPECIFICATIONS	RESULTS
Phenotypic Analysis		
Cellular morphology	Gram-positive rod	Gram-positive rod
Colony morphology ²	Report results	Slightly irregular, raised, opaque and grey (Figure 1)
Sporulation	Positive	Positive
Motility	Motile	Motile
β-hemolysis	Hemolytic	Hemolytic
Capsule (India ink staining)	Negative	Negative
Tenacious	Report results	Positive
Analytical profile index (API® 50 CHB/API® 20E)	Consistent with Bacillus cereus	Consistent with <i>Bacillus</i> sp. ³
Nitrate reduction	Positive	Positive
Genotypic Analysis Sequencing of 16S ribosomal RNA gene (~ 1400 base pairs)	Consistent with <i>B. cereus</i> group	Consistent with <i>B. cereus</i> group ⁴
PCR Assay of Extracted DNA ⁵		
gyrB ⁶	~ 475 bp amplicon (<i>B. cereus</i>)	~ 475 bp amplicon (B. cereus)
groEL	~ 400 bp amplicon (<i>B. cereus</i> group)	~ 400 bp amplicon (<i>B. cereus</i> group)
sspE ⁷	~ 70 bp amplicon (<i>B. cereus</i> group)	~ 70 bp amplicon (<i>B. cereus</i> group)
Viability (post-freeze) ²	Growth	Growth

¹NR-12151 was produced by inoculation of Tryptic Soy Broth with the deposited material and grown 24 hours at 37°C. Broth inoculum was added to Kolles which were grown 24 hours at 37°C to produce this lot.

Figure 1



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²24 hours at 37°C and aerobic atmosphere on Tryptic Soy Agar with 5% sheep blood

³The API® 50 CHB/API® 20E results could only determine that NR-12151 was from the genus *Bacillus*. Additional PCR analysis was completed to verify NR-12151 as *B. cereus*.

⁴Bacillus cereus group species (*B. cereus, B. thuringiensis, B. mycoides*, and *B. anthracis*) cannot be classified based on 16S sequence (Spencer, R. C. "Bacillus anthracis." J. Clin. Pathol. 56 (2003): 182-187. PubMed: 12610093).

⁵All genes assayed were chromosomally located

⁶PCR amplification of the *gyrB* gene yields a 253 bp amplicon for *B. anthracis*, a 604 bp amplicon for *B. mycoides* and a 737 bp amplicon for *B. thuringiensis*. For additional PCR information see Park, S.-H. et al. "Simultaneous Detection and Identification of *Bacillus cereus* Group Bacteria Using Multiplex PCR." J. Microbiol. Biotechnol. 17 (2007): 1177-1182. PubMed: 18051330.

⁷PCR amplification of the *sspE* gene yields two amplicons for *B. anthracis* that are a 188 bp and 70 bp. Other *B. cereus* group species show only the 70 bp amplicon and non-*B. cereus* group species show no amplicons.



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Date: 03 JUN 2011

Signature:

Title:

Technical Manager, BEI Authentication or designee

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