

Certificate of Analysis for NR-22135

Bacillus cereus, Strain VD078

Catalog No. NR-22135

Product Description: Bacillus cereus (B. cereus), strain VD078 was isolated in 2008 from a soil sample collected in Greenland.

Lot¹: 61956002 Manufacturing Date: 15AUG2013

TEST	SPECIFICATIONS	RESULTS
Phenotypic Analysis ²		N.20210
Cellular morphology	Gram-positive rods	Gram-positive rods
Colony morphology ³	Report results	Circular, raised, entire, opaque, ground-
Colorly morphology	Report results	glass and cream (Figure 1)
Motility ⁴	Motile	Motile
Hemolysis ⁵	Report results	β-hemolytic
Biochemical tests ⁶		p
Production of acid from trehalose	Positive	Positive
Production of acid from salicin ⁷	Report results	Positive
Production of acid from glycerol ⁷	Report results	Positive
Nitrate reduction	Report results	Positive
Arginine dihydrolase activity ⁸	Report results	Negative
PCR Assay of Extracted DNA		
16S ribosomal RNA gene	~ 560 bp amplicon	~ 560 bp amplicon
B. anthracis specific chromosomal marker9	No amplicon	No amplicon
Presence of virulence plasmids ¹⁰	·	·
pXO1 (four targets)	No amplicons	No amplicons
pXO2 (three targets)	Report results	No amplicons
Genotypic Analysis		
Sequencing of 16S ribosomal RNA gene	Consistent with B. cereus group	Consistent with <i>B. cereus</i> group 11,12
(~ 870 base pairs)		
Purity (post-freeze) ¹³	Growth consistent with B. cereus	Growth consistent with B. cereus
Viability (post-freeze) ³	Growth	Growth

¹The deposited material was inoculated into Tryptic Soy broth and grown at 37°C for 24 hours in an aerobic atmosphere, and the resulting subculture was vialed and frozen. NR-22135 was produced by inoculation of the thawed subculture into Tryptic Soy broth and grown 21 hours at 37°C in an aerobic atmosphere. Broth inoculum was added to Tryptic Soy agar kolles which were grown 21 hours at 37°C in an aerobic atmosphere to produce this lot.

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²Presumptive identification of *B. cereus* was performed using phenotypic tests that eliminate other *B. cereus* group (*B. cereus*, *B. anthracis*, *B.* thuringiensis and B. mycoides) members (see footnotes 4, 7 and 8).

³23 hours at 37°C in an aerobic atmosphere on Tryptic Soy agar

⁴24 hours at 37°C in an aerobic atmosphere on motility test media with triphenyltetrazolium chloride (TTC). In the *B. cereus* group, *B. cereus* and *B.* thuringiensis are motile, whereas B. anthracis and B. mycoides are non-motile.

⁵24 hours at 37°C in an aerobic atmosphere on Tryptic Soy agar with 5% sheep blood

⁶Negative tests are observed for >7 days.

⁷B. anthracis is negative for glycerol and salicin.

⁸B. thuringiensis is positive for arginine dihydrolase activity.

⁹A proprietary (Patent Pending) PCR-based assay capable of differentiating B. anthracis from the remainder of the B. cereus group was used to further eliminate B. anthracis as a possible species.

¹⁰Presence of virulence plasmids was verified using a proprietary (Patent Pending) PCR-based assay. NR-22135 is reported by the depositor to contain a pXO1-like plasmid. It is not known if the targets assayed are present.

¹¹B. cereus group species cannot be classified based on 16S sequence (Spencer, R. C. "Bacillus anthracis." J. Clin. Pathol. 56 (2003): 182-187. PubMed: 12610093).

¹²≥ 99.7% identical to *B. cereus*, strain VD078 (GenBank: AHEV01000034.1)

¹³Purity of this lot was assessed for 7 days on Tryptic Soy agar at 37°C in an aerobic atmosphere.



Certificate of Analysis for NR-22135

Figure 1



Date: 16 AUG 2014

Signature:

Title: Technical Manager, BEI Authentication or designee

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