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SUPPORTING INFECTIOUS DISEASE RESEARCH

Certificate of Analysis for NR-22150

Bacillus cereus, Strain VD148

Catalog No. NR-22150

Product Description: *Bacillus cereus* (*B. cereus*), strain VD148 was isolated in 2008 from a soil sample collected in Scotland, United Kingdom.

Lot[1,2]: 61660058 **Manufacturing Date: 03APR2013**

TEST	SPECIFICATIONS	RESULTS
Phenotypic Analysis[3]		
Cellular morphology	Gram-positive rods	Gram-positive rods
Colony morphology[4]	Report results	Circular, low convex, rough and gray-brown (Figure 1)
Motility[5]	Motile	Motile
Hemolysis	Report results	β-hemolytic
Biochemical characterization[6]		
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	Report results	Positive
PCR Assay of Extracted DNA		
16S ribosomal RNA gene	~ 560 bp amplicon	~ 560 bp amplicon
B. anthracis specific chromosomal marker[8]	No amplicon	No amplicon
Presence of virulence plasmids[9]		
pXO1 (four targets)	No amplicons	No amplicons
pXO2 (three targets)	Report results	No amplicons
Genotypic Analysis		
Sequencing of 16S ribosomal RNA gene (~ 1490 base pairs)	Consistent with *B. cereus* group	Consistent with *B. cereus* group[10,11]
Purity (post-freeze)[12]	Growth consistent with *B. cereus*	Growth consistent with *B. cereus*
Viability (post-freeze)[4]	Growth	Growth

[1]The deposited material was inoculated into Tryptic Soy broth and kept in an aerobic atmosphere at 37°C for 24 hours; no growth was observed. Broth inoculum was added to Tryptic Soy agar plates, held for 24 hours at 37°C in an aerobic atmosphere, and the resulting subculture vialed and frozen. NR-22150 was produced by inoculation of the thawed subculture into Tryptic Soy broth and grown 24 hours at 37°C in an aerobic atmosphere. Broth inoculum was added to Tryptic Soy broth with 5% sheep blood kolles which were grown 24 hours at 37°C in an aerobic atmosphere to produce this lot.

[2]NR-22150 was deposited as *B. cereus*. Current quality control testing at ATCC® could not distinguish between *B. cereus* and *B. thuringiensis* for this product.

[3]Presumptive 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 2, 5, 7 and 8).

[4]24 hours at 37°C in an aerobic atmosphere on Tryptic Soy agar with 5% sheep blood

[5]24 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.

[6]Negative tests are observed for >7 days.

[7]*B. anthracis* is negative for glycerol and salicin.

[8]A 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.

[9]Presence of virulence plasmids was verified using a proprietary (Patent Pending) PCR-based assay. NR-22150 is reported by the depositor to contain a pXO2-like plasmid. It is not known if the targets assayed are present.

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

[11]100% identical to *B. cereus*, strain VD148 (GenBank: AHFF01000054.1)

[12]Purity of this lot was assessed for 7 days on Tryptic Soy agar with 5% sheep blood at 37°C in an aerobic atmosphere.

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Certificate of Analysis for NR-22150

Figure 1

Date: 20 AUG 2014 **Signature:**

Title: Technical Manager, BEI Authentication or designee

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