

## **Certificate of Analysis for NR-22148**

## Bacillus cereus, Strain VD115

## Catalog No. NR-22148

Product Description: Bacillus cereus (B. cereus), strain VD115 was isolated in 2008 from a soil sample collected in Guadeloupe, French West Indies.

Lot<sup>1</sup>: 61660057 Manufacturing Date: 03APR2013

TEST	SPECIFICATIONS	RESULTS
Phenotypic Analysis <sup>2</sup>		
Cellular morphology	Gram-positive rods	Gram-positive rods
Colony morphology <sup>3</sup>	Report results	Circular, umbonate, slight undulate, rough and gray (Figure 1)
Motility <sup>4</sup>	Motile	Motile
Hemolysis	Report results	β-hemolytic
Biochemical tests <sup>5</sup>		·
Production of acid from trehalose	Positive	Positive
Production of acid from salicin <sup>6</sup>	Report results	Positive
Production of acid from glycerol <sup>6</sup>	Report results	Positive
Nitrate reduction	Report results	Positive
Arginine dihydrolase activity <sup>7</sup>	Report results	Negative
PCR Assay of Extracted DNA		
16S ribosomal RNA gene	~ 560 bp amplicon	~ 560 bp amplicon
B. anthracis specific chromosomal marker <sup>8</sup>	No amplicon	No amplicon
Presence of virulence plasmids <sup>9</sup>		
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 10,11
(~ 1510 base pairs)		
Purity (post-freeze) <sup>12</sup>	Growth consistent with B. cereus	Growth consistent with B. cereus
Viability (post-freeze) <sup>3</sup>	Growth	Growth

The deposited material was inoculated into Tryptic Soy broth and grown at 37°C for 24 hours in an aerobic atmosphere, and the resulting subculture vialed and frozen. NR-22148 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.

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<sup>&</sup>lt;sup>2</sup>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 5, 7 and 8).

<sup>&</sup>lt;sup>3</sup>24 hours at 37°C in an aerobic atmosphere on Tryptic Soy agar with 5% sheep blood

<sup>&</sup>lt;sup>4</sup>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.

<sup>&</sup>lt;sup>5</sup>Negative tests are observed for >7 days.

<sup>&</sup>lt;sup>6</sup>B. anthracis is negative for glycerol and salicin.

<sup>&</sup>lt;sup>7</sup>B. thuringiensis is positive for arginine dihydrolase activity.

<sup>&</sup>lt;sup>8</sup>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.

<sup>&</sup>lt;sup>9</sup>Presence of virulence plasmids was verified using a proprietary (Patent Pending) PCR-based assay. NR-22148 is reported by the depositor to contain a pXO2-like plasmid. It is not known if the targets assayed are present.

<sup>&</sup>lt;sup>10</sup>B. cereus group species cannot be classified based on 16S séquence (Spencer, R. C. "Bacillus anthracis." <u>J. Clin. Pathol.</u> 56 (2003): 182-187. PubMed: 12610093).

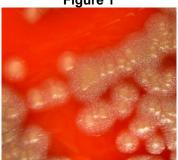
<sup>&</sup>lt;sup>11</sup>≥ 99.9% identical to *B. cereus*, strain VD115 (GenBank: AHEY01000037.1)

<sup>&</sup>lt;sup>12</sup>Purity of this lot was assessed for 7 days on Tryptic Soy agar at 37°C in an aerobic atmosphere.



## **Certificate of Analysis for NR-22148**

Figure 1



Date: 20 AUG 2014

Signature:

Title: Technical Manager, BEI Authentication or designee

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