

## Certificate of Analysis for NR-28575

## Bacillus cereus, Strain BAG1X1-1

## Catalog No. NR-28575

**Product Description:** Bacillus cereus (B. cereus), strain BAG1X1-1 was isolated in 2009 from a soil sample collected in Boston, Massachusetts, USA.

Lot<sup>1</sup>: 61317363 Manufacturing Date: 07NOV2012

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, erose, rough and gray (Figure 1)
Motility <sup>4</sup>	Motile	Motile
Hemolysis	Report results	β-hemolytic
Biochemical characterization <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 <sup>8</sup>		
16S ribosomal RNA gene	~ 560 bp amplicon	~ 560 bp amplicon
B. anthracis specific chromosomal marker <sup>9</sup>	No amplicon	No amplicon
Presence of virulence plasmid markers <sup>10</sup>	<u>'</u>	'
pXO1 (four targets)	Report results	One amplicon present
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 <sup>11</sup>
(~ 1450 base pairs)		
Viability (post-freeze) <sup>3</sup>	Growth	Growth

<sup>&</sup>lt;sup>1</sup>NR-28575 was produced by inoculation of Nutrient broth with the deposited material and grown 24 hours at 30°C in an aerobic atmosphere. After an additional passage under the above propagation conditions, broth inoculum was added to Tryptic Soy agar with 5% sheep blood kolles which were grown 24 hours at 30°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 4, 6, 7).

<sup>&</sup>lt;sup>3</sup>24 hours at 30°C in an aerobic atmosphere on Tryptic Soy agar with 5% sheep blood

<sup>&</sup>lt;sup>4</sup>24 hours at 30°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 were 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.

DNA was extracted from a broth culture produced from NR-28575 lot 61317363.

<sup>&</sup>lt;sup>9</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>10</sup>Presence of markers known to be found on virulence plasmids were verified using a proprietary (Patent Pending) PCR-based assay.

<sup>&</sup>lt;sup>11</sup>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).



## **Certificate of Analysis for NR-28575**





**Date:** 14 MAR 2014

Signature:

**Title:** Technical Manager, BEI Authentication or designee

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