

Certificate of Analysis for NR-28599

Bacillus cereus, Strain BAG4X2-1

Catalog No. NR-28599

Product Description: Bacillus cereus (B. cereus), strain BAG4X2-1 was isolated in 2008 from a soil sample collected in Boston, Massachusetts, USA.

Lot^{1,2}: 61646461 Manufacturing Date: 27MAR2013

TEST	SPECIFICATIONS	RESULTS
Phenotypic Analysis ³		
Cellular morphology	Gram-positive rods	Gram-positive rods
Colony morphology ⁴	Report results	Circular, entire, opaque and rough (Figure 1)
Motility ⁵	Motile	Motile
Hemolysis	Report results	β-hemolytic
Biochemical tests 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	Consistent with B. cereus group	Consistent with B. cereus group 10,11
(~ 960 base pairs)	-	
Purity (post-freeze) ¹²	Growth consistent with B. cereus	Growth consistent with B. cereus
Viability (post-freeze) ⁴	Growth	Growth

The deposited material was passaged three times on Tryptic Soy agar at 30°C for 24 hours in an aerobic atmosphere, and the resulting subculture vialed and frozen. NR-28599 was produced by inoculation of the thawed subculture into Tryptic Soy broth and grown 24 hours at 30°C in an aerobic atmosphere. Broth inoculum was added to Tryptic Soy broth with 5% sheep blood kolles which were grown 24 hours at 30°C in an aerobic atmosphere to produce this lot.

BEI Resources

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²NR-28599 was deposited as *B. cereus*. Current quality control testing at ATCC could not distinguish between *B. cereus* and *B. thuringiensis* for this product.

³Presumptive 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).

⁴24 hours at 30°C in an aerobic atmosphere on Tryptic Soy agar with 5% sheep blood

⁵24 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.

⁶Negative tests are observed for >7 days.

⁷B. anthracis is negative for glycerol and salicin.

⁸A 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.

⁹Presence of virulence plasmids was verified using a proprietary (Patent Pending) PCR-based assay. NR-28599 is reported by the depositor to contain a pXO2-like plasmid. It is not known if the targets assayed are present.

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

¹¹99.9% identical to *B. cereus*, strain BAG4X2-1 (GenBank: AHDH01000011.1)

¹²Purity of this lot was assessed for 7 days on Tryptic Soy agar with 5% sheep blood at 30°C in an aerobic atmosphere.



Certificate of Analysis for NR-28599

Figure 1



Date: 20 AUG 2014

Signature:

Title: Technical Manager, BEI Authentication or designee

ATCC®, on behalf of BEI Resources, hereby represents and warrants that the material provided under this certificate has been subjected to the tests and procedures specified and that the results described, along with any other data provided in this certificate, are true and accurate to the best of ATCC®'s knowledge.

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