Certificate of Analysis for NR-28582

Bacillus cereus, Strain BAG1O-2

Catalog No. NR-28582

Product Description: *Bacillus cereus* (*B. cereus*), strain BAG1O-2 was isolated in 2009 from a soil sample collected in Boston, Massachusetts, USA.

Lot[1,2]: 61646458 **Manufacturing Date: 04APR2013**

TEST	SPECIFICATIONS	RESULTS
Phenotypic Analysis[3]		
Cellular morphology	Gram-positive rods	Gram-positive rods
Colony morphology[4]	Report results	Irregular, low convex, ground-glass and beige (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[8]		
16S ribosomal RNA gene	~ 560 base pair amplicon	~ 560 base pair amplicon
B. anthracis specific chromosomal marker[9]	No amplicon	No amplicon
Presence of virulence plasmids[10]		
pXO1 (four targets)	No amplicons	No amplicons
pXO2 (three targets)	No amplicons	No amplicons
Genotypic Analysis		
Sequencing of 16S ribosomal RNA gene (~ 840 base pairs)	Consistent with *B. cereus* group	Consistent with *B. cereus* group[11]
Viability (post-freeze)[4]	Growth	Growth

[1]NR-28582 was produced by inoculation of the deposited material into Tryptic Soy broth and grown for 1 day at 37°C in an aerobic atmosphere. Broth inoculum was added to Tryptic Soy broth with 5% defibrinated sheep blood kolles, which were grown for 1 day at 37°C in an aerobic atmosphere to produce this lot.
[2]NR-28582 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 accomplished using phenotypic tests that eliminate other *B.* cereus group (*B. cereus, B. anthracis, B. thuringiensis* and *B. mycoides*) members (see footnotes 2, 5, 7, 8).
[4]1 day at 37°C in an aerobic atmosphere on Tryptic Soy agar with 5% defibrinated sheep blood
[5]1 day 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]DNA was extracted from a broth culture produced from NR-28582 lot 61646458.
[9]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.
[10]Presence of virulence plasmids was verified using a proprietary (Patent Pending) PCR-based assay.
[11]*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).

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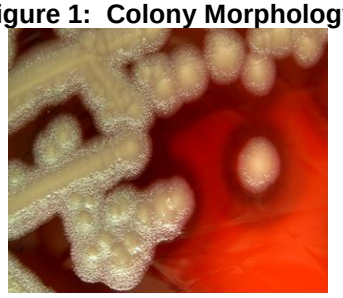
Figure 1: Colony Morphology



Date: 20 MAR 2017 **Signature:**

BEI Resources Authentication

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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