b|e|i RESOURCES
SUPPORTING INFECTIOUS DISEASE RESEARCH

Certificate of Analysis for NR-28578

Bacillus cereus, Strain BAG1X2-1

Catalog No. NR-28578

Product Description: *Bacillus cereus* (*B. cereus*), strain BAG1X2-1 was isolated in 2009 from a soil sample collected in Boston, Massachusetts, USA.

Lot[1]: 61317365 **Manufacturing Date: 09NOV2012**

TEST	SPECIFICATIONS	RESULTS
Phenotypic Analysis[2]		
Cellular morphology	Gram-positive rods	Gram-positive rods
Colony morphology[3]	Report results	Circular, umbonate, erose and gray (Figure 1)
Motility[4]	Motile	Motile
Hemolysis	Report results	β-hemolytic
Biochemical characterization[5]		
Production of acid from trehalose	Positive	Positive
Production of acid from salicin[6]	Report results	Positive
Production of acid from glycerol[6]	Report results	Positive
Nitrate reduction	Report results	Positive
Arginine dihydrolase activity[7]	Report results	Negative
PCR Assay of Extracted DNA[8]		
16S ribosomal RNA gene	~ 560 bp amplicon	~ 560 bp amplicon
B. anthracis specific chromosomal marker[9]	No amplicon	No amplicon
Presence of virulence plasmid markers[10]		
pXO1 (four targets)	Report results	One amplicon present
pXO2 (three targets)	Report results	No amplicons
Genotypic Analysis		
Sequencing of 16S ribosomal RNA gene (~ 930 base pairs)	Consistent with *B. cereus* group	Consistent with *B. cereus* group[11]
Viability (post-freeze)[3]	Growth	Growth

[1]NR-28578 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.
[2]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).
[3]24 hours at 30°C in an aerobic atmosphere on Tryptic Soy agar with 5% sheep blood
[4]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.
[5]Negative tests were observed for >7 days.
[6]*B. anthracis* is negative for glycerol and salicin.
[7]*B. thuringiensis* is positive for arginine dihydrolase activity.
[8]DNA was extracted from a broth culture produced from NR-28578 lot 61317365.
[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 markers known to be found on virulence plasmids were 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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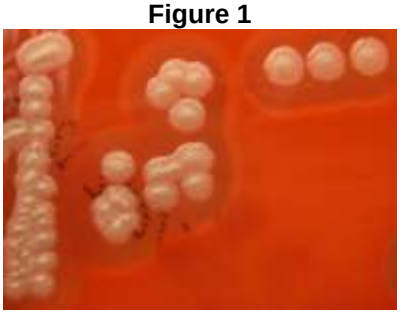
Figure 1



Date: 14 MAR 2014

Signature:

Title: Technical Manager, BEI Authentication or designee

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