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Certificate of Analysis for NR-9999

Bacillus anthracis, Strain Sterne ΔGBAA1346

Catalog No. NR-9999

Product Description: *Bacillus anthracis* (*B. anthracis*), strain Sterne ΔGBAA1346 is a markerless, nonpolar, deletion mutant of the toxigenic acapsulate original Sterne strain (34F2). Nearly the entire open reading frame is replaced by three stop codons followed by two restriction endonuclease recognition sites, *Bam*HI and *Sma*I (to facilitate screening of the correct mutation). The first and last ten codons of the putative internalin (GBAA1346) gene retain the wild type sequence.

Lot[1]: 58394759 **Manufacturing Date: 07NOV2008**

TEST	SPECIFICATIONS	RESULTS
Phenotypic Analysis		
Cellular morphology	Gram-positive rod	Gram-positive rod
Colony morphology		
Tryptic Soy Agar, 5% sheep blood[2]	Report results	Circular, low convex, erose, ground-glass, opaque and grey (Figure 1)
PLET Agar[2,3]	Report results	Circular, flat, lobate, ground-glass, opaque and cream (Figure 2)
Sporulation	Positive	Positive
Motility	Non-motile	Non-motile
β-hemolysis	Non-hemolytic	Non-hemolytic
Capsule (India ink staining)	Negative	Negative
Tenacious	Positive	Positive
Analytical profile index (API® 50 CHB including API® 20E; ONPG to GEL only)	Consistent with *B. anthracis*	Consistent with *B. anthracis*
Nitrate reduction	Positive	Positive
Genotypic Analysis		
Sequencing of 16S ribosomal RNA gene (1450 base pairs)	Consistent with *Bacillus cereus* group	Consistent with *Bacillus cereus* group[4]
PCR Assay of Extracted DNA		
16S ribosomal RNA gene	~ 1500 bp amplicon	~ 1500 bp amplicon
Presence of virulence plasmids		
pXO1 (*aat*)	~ 125 bp amplicon	~ 125 bp amplicon
pXO2 (*at*, *capA, capB, capC*)	No amplicons	No amplicons
Viability (post-vialing)[5]	Growth	Growth

[1] *B. anthracis*, strain Sterne ΔGBAA1346 was deposited by Philip C. Hanna, Associate Professor, Department of Microbiology and Immunology, University of Michigan Medical School, Ann Arbor, Michigan. NR-9999 was produced by inoculation of the deposited material into Tryptic Soy Broth and grown 24 hours at 37°C. Broth inoculum was added to Kolles which were grown 24 hours at 37°C to produce this lot.

[2] 24 hours at 37°C

[3] Growth on PLET [polymyxin-lysozyme-EDTA-thallous acetate] Agar (Hardy Diagnostics, Cat. No. G153) differentiates *B. anthracis* from other *Bacillus* species, including *B. cereus*, *B. thuringiensis* and *B. mycoides*, whose growth is inhibited by the combination of EDTA and thallium cations. Dragon, D. C. and R. P. Rennie. "Evaluation of Spore Extraction and Purification Methods for Selective Recovery of Viable *Bacillus anthracis* Spores." Lett. Appl. Microbiol. 33 (2001): 100-105. PubMed: 11472515.

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

[5] 24 hours at 37°C in Tryptic Soy Broth

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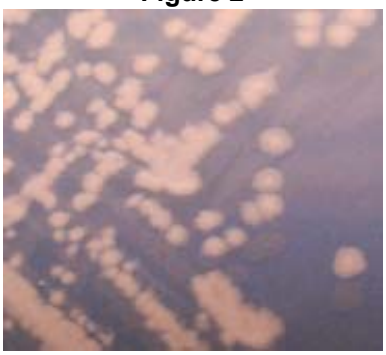
Figure 1

Figure 2



Date: 23 JUN 2009

Signature: Signature on File

Title: Technical Manager, BEI Authentication or designee

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