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SUPPORTING INFECTIOUS DISEASE RESEARCH

Certificate of Analysis for NR-9399

Bacillus anthracis, Strain Sterne BA690 (Δ*pagA242*)

Catalog No. NR-9399

This reagent is the tangible property of the U.S. Government.

Product Description: *Bacillus anthracis* (*B. anthracis*), strain Sterne BA690 (Δ*pagA242*) is a 2,247 base pair deletion mutant of the toxigenic acapsulate Sterne 7702 strain. The designation BA690 refers to the numbering system used in the Stibitz laboratory. The presence of pXO1 (but absence of *pag*) and the absence of pXO2 in NR-9399 have been confirmed by PCR amplification of plasmid-specific sequences from extracted DNA (see Table below).

Lot[1,2]: 57969328 **Manufacturing Date: 28NOV2008**

TEST	SPECIFICATIONS	RESULTS
Phenotypic Analysis		
Cellular morphology	Gram-positive rod	Gram-positive rod
Colony morphology[3]	Report results	Flat, entire, ground-glass, grey (Figure 1)
Sporulation	Positive	None observed[4]
Motility	Non-motile	Non-motile
β-hemolysis	Non-hemolytic	Non-hemolytic
Capsule (India ink staining)[5]	Negative	Negative
Tenacious	Positive	Positive
Analytical profile index (API® 50 CHB/API® 20E)	Consistent with *B. anthracis*	Consistent with *B. anthracis*
Nitrate reduction	Positive	Positive
FAME analysis	Consistent with *B. anthracis*	Consistent with *B. anthracis*
Genotypic Analysis		
Sequencing of 16S ribosomal RNA gene (~ 1420 base pairs)	Consistent with *Bacillus cereus* group	Consistent with *Bacillus cereus* group[6]
PCR Assay of Extracted DNA		
16S ribosomal RNA gene	~ 555 bp amplicon	~ 555 bp amplicon
*S*pecific chromosomal marker[7]	Amplicon present	Amplicon present
Presence of virulence plasmids[8]		
pXO1 (three targets)	Amplicons present	Amplicons present
pXO2 (three targets)	No amplicons	No amplicons
Verification of Δ*pag*	No amplicon	No amplicon
Viability (post-vialing)[3]	Growth	Growth

[1] *B. anthracis*, strain Sterne BA690 (Δ*pagA242*) was deposited by E. Scott Stibitz, Division of Bacterial, Parasitic, and Allergenic Products, Center for Biologics Evaluation and Research, Food and Drug Administration, Bethesda, Maryland. NR-9399 was produced by inoculation of the deposited material into Tryptic Soy Broth and grown 24 hours at 37°C and 5% CO_2. Broth inoculum was added to Kolles which were grown 24 hours at 37°C and 5% CO_2 to produce this lot.

[2] A portion of this lot underwent brief thawing after being vialed. All Certificate of Analysis tests were performed on vials that had been thawed and those that were not. The results obtained from the thawed vials were identical to the results obtained from the vials that were not thawed.

[3] 24 hours at 37°C and 5% CO_2 on Tryptic Soy Agar with 5% sheep blood

[4] Spores are not present unless exposed to low CO_2 levels, such as those found in the ambient atmosphere. Higher CO_2 levels inhibit spore formation.

[5] Virulent strains are positive for encapsulation.

[6] *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).

[7] This product was verified to a species level using a proprietary (Patent Pending) PCR-based assay to a *Bacillus anthracis*-specific genetic mutation capable of differentiating *B. anthracis* from the remainder of the *B. cereus* group.

[8] Plasmids were verified using a proprietary (Patent Pending) PCR-based assay to a *Bacillus anthracis*-plasmids pXO1 and pXO2.

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Certificate of Analysis for NR-9399

Figure 1

Date: 04 SEP 2012

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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