

Certificate of Analysis for NR-52261

Bacillus paranthracis, Strain PCI 246

Catalog No. NR-52261

(Derived from ATCC® 13061™)

Product Description:

Bacillus paranthracis (B. paranthracis), strain PCI 246 was originally deposited at ATCC® as B. cereus Frankland and Frankland. NR-52261 lot 70033094 was produced by inoculation of ATCC® 13061™ lot 70012155 into Tryptic Soy broth and grown for 1 day at 30°C in an aerobic atmosphere. Broth inoculum was added to Tryptic Soy agar kolles, which were grown for 1 day at 30°C in an aerobic atmosphere to produce this lot. Quality control testing was completed under propagation conditions unless otherwise noted.

Lot: 70033094 Manufacturing Date: 26FEB2020

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TEST	SPECIFICATIONS	RESULTS
Phenotypic Analysis		
Cellular morphology	Gram-positive rods	Gram-positive rods
Colony morphology	Report results	Irregular, convex, lobate, smooth, opaque and cream (Figure 1)
Hemolysis 1 day at 37°C in an aerobic atmosphere on Tryptic Soy agar with 5% defibrinated sheep blood	Report results	β-hemolytic
Motility (wet mount) Biochemical tests	Report results	Motile
Catalase	Report results	Negative
VITEK® MS (MALDI-TOF)	B. cereus group	B. cereus group (99.9%) ¹
Genotypic Analysis		
Digital DNA-DNA hybridization (dDDH) ²	> 70% dDDH value for identity to B. cereus	B. paranthracis (77.3%) ^{3,4}
Presence of <i>B. anthracis</i> virulence plasmids		
pXO1	Absence of sequence confirmed	Absence of sequence confirmed
pXO2	Absence of sequence confirmed	Absence of sequence confirmed
Purity (post-freeze) 7 days at 37°C in an aerobic atmosphere with 5% CO ₂ on Tryptic Soy agar with 5% defibrinated sheep blood	Growth consistent with expected colony morphology	Growth consistent with expected colony morphology
Viability (post-freeze)	Growth	Growth

VITEK® MS (MALDI-TOF) was used to confirm to genus. Due to high protein sequence similarities between members of the *B. cereus* group, identification of a single species can not be confirmed using standard MALDI-TOF databases. For additional information, refer to Ha, M., et al. "Reliable Identification of *Bacillus cereus* Group Species Using Low Mass Biomarkers by MALDI-TOF MS." <u>J. Microbiol. Biotechnol.</u> 29 (2019): 887-896. PubMed: 31216842.

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²Relatedness between bacterial strains has traditionally been determined using DDH. For additional information, refer to Auch, A. F., et al. "Digital DNA-DNA Hybridization for Microbial Species Delineation by Means of Genome-to-Genome Sequence Comparison." <u>Stand. Genomic Sci.</u> 2 (2010): 117-134. PubMed: 21304684. dDDH analysis was performed using the Type (Strain) Genome Server.

³The whole genome of *B. paranthracis*, strain PCI 246 was sequenced using the Illumina MiSeq system. *De novo* contig sequences were generated using Unicycler v0.4.8-beta.

⁴The closest matching type strain is *B. paranthracis*, strain MCC1A00395 with a dDDH value of 77.3%.



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Figure 1: Colony Morphology



/Heather Couch/ Heather Couch

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Program Manager or designee, ATCC Federal Solutions

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