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SUPPORTING INFECTIOUS DISEASE RESEARCH

Certificate of Analysis for NR-52260

Bacillus cereus, Strain NRRL B-569

Catalog No. NR-52260
(Derived from ATCC® 10876™)

Product Description:
Bacillus cereus (B. cereus), strain NRRL B-569 was isolated in 1944 from a contaminated flask. NR-52260 lot 70033092 was produced by inoculation of ATCC® 10876™ lot 70009511 into Nutrient broth and grown for 1 day at 30°C in an aerobic atmosphere. Broth inoculum was added to Nutrient 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: 70033092 **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, flat, entire, undulate, rough and cream (Figure 1)
Hemolysis 1 day at 30°C in an aerobic atmosphere on Tryptic Soy agar with 5% defibrinated sheep blood	Report results	β-hemolytic
Motility (wet mount)	Report results	Motile
Biochemical tests		
Catalase	Report results	Positive
VITEK® MS (MALDI-TOF)	*B. cereus* group	*B. cereus* group (99.9%)[1]
Genotypic Analysis		
Sequencing of 16S ribosomal RNA gene (~ 1490 base pairs)	≥ 99% sequence identity to *B. cereus*, strain NRR: B-569 (GenBank: ACLT01000002.1)	99.9% sequence identity to *B. cereus*, strain NRR: B-569 (GenBank: ACLT01000002.1)[2]
Digital DNA-DNA hybridization (dDDH)[3]	≥ 70% for species identification	*B. cereus* (72.5%)[4]
Presence of *B. anthracis* 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 Nutrient agar	Growth consistent with expected colony morphology	Growth consistent with expected colony morphology
Viability (post-freeze)	Growth	Growth

[1]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." J. Microbiol. Biotechnol. 29 (2019): 887-896. PubMed: 31216842.

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

[3]Relatedness 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." Stand. Genomic Sci. 2 (2010): 117-134. PubMed: 21304684. dDDH analysis was performed using the Type (Strain) Genome Server.

[4]The whole genome of *Bacillus cereus*, strain NRRL B-569 was sequenced using the Illumina® MiSeq® system. *De novo* contig sequences were generated using Unicycler v0.4.8-beta.

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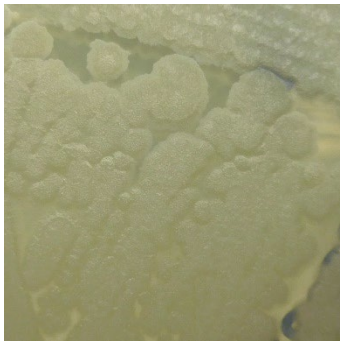
Figure 1: Colony Morphology



/Heather Couch/
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Program Manager or designee, ATCC Federal Solutions

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