Certificate of Analysis for NR-52256

Bacillus sp., Strain NRS 201

Catalog No. NR-52256
(Derived from ATCC® 7064™)

Product Description:
Bacillus sp., strain NRS 201 was originally isolated from blood by L. Siribaed and deposited at ATCC® by Dr. N. R. Smith as *Bacillus siamensis* Siribaed. NR-52256 lot 70033084 was produced by inoculation of ATCC® 7064™ lot 4987158 into Tryptic Soy broth and grown for 1 day at 25°C in an aerobic atmosphere. Broth inoculum was added to Tryptic Soy agar with 5% defibrinated sheep blood kolles, which were grown for 1 day at 25°C in an aerobic atmosphere to produce this lot. Quality control testing was completed under propagation conditions unless otherwise noted.

Lot: 70033084 **Manufacturing Date: 12FEB2020**

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TEST	SPECIFICATIONS	RESULTS
Phenotypic Analysis		
Cellular morphology	Gram-positive rods	Gram-positive rods
Colony morphology	Report results	Circular, low convex, undulate, opaque, ground-glass and gray (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)	Report results	Motile
Biochemical tests		
Catalase	Report results	Positive
VITEK® MS (MALDI-TOF)	*Bacillus cereus* group	*Bacillus cereus* group (99.9%)[1]
Genotypic Analysis		
Digital DNA-DNA hybridization (dDDH)[2]	> 70% dDDH value for identity to *Bacillus cereus*	< 70% dDDH value for identity to any *Bacillus* type species[3,4]
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 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

[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]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.

[3]The whole genome of *Bacillus* sp., strain NRS 201 was sequenced using the Illumina® MiSeq® system. *De novo* contig sequences were generated using Unicycler v0.4.8-beta.

[4]The closest matching type strain is *B. paranthracis,* strain MCC1A00395 with a dDDH value of 61.3%. This result suggests that NR-52256 may represent a new *Bacillus* species.

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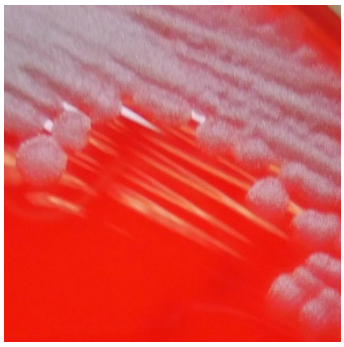
Figure 1: Colony Morphology



/Heather Couch/
Heather Couch 05 MAY 2021
Program Manager or designee, ATCC Federal Solutions

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