Certificate of Analysis for NR-44265

Mycobacterium avium subsp. *avium*, Strain 2285 Smooth

Catalog No. NR-44265

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Product Description:

Mycobacterium avium (M. avium) subsp. *avium,* strain 2285 Smooth was isolated between 2009 and 2013 from human sputum at the National Institute of Allergy and Infectious Diseases (NIAID), National Institutes of Health (NIH), Bethesda, Maryland, USA. NR-44265 was deposited to BEI Resources as *M. avium*. Whole genome sequencing performed by BEI Resources identified strain 2285 Smooth as subsp. *avium*. NR-44265 was produced by inoculation of BEI Resources seed lot 62009737 into Middlebrook 7H9 broth with ADC enrichment and grown for 14 days at 37°C in an aerobic atmosphere with 5% CO_2. Broth inoculum was added to Middlebrook 7H10 agar with OADC enrichment kolles, which were grown for 14 days at 37°C in an aerobic atmosphere with 5% CO_2 to produce this lot. Quality control testing was completed under propagation conditions unless otherwise noted.

Lot: 70062660 **Manufacturing Date: 08SEP2023**

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TEST	SPECIFICATIONS	RESULTS
Phenotypic Analysis[1]		
Cellular morphology	Gram-positive rods	Gram-positive rods
Colony morphology	Report results	Circular, convex, entire, smooth and cream (Figure 1)
Motility (wet mount)	Report results	Non-motile
Growth rate	≥ 7 days	14 days
Growth at 37°C	Positive	Positive
Growth at 45°C	Positive	Negative[2]
Growth at 55°C	Negative	Negative
Acid-fast stain	Positive (red colonies)	Positive (red colonies)
Pigmentation in the dark (Scotochromogen)	Negative (no pigment)	Negative (no pigment)
Photoinduction for 1 hour (Photochromogen)	Negative	Negative
Nonchromogen (no pigment)	Positive	Positive
VITEK® MS (MALDI-TOF)	*M. avium*	*M. avium* (99.9%)
Biochemical tests[3]		
Catalase	Positive	Positive
Catalase (68°C)	Negative	Negative
Iron uptake	Negative	Positive[2]
Nitrate reduction	Negative	Negative
Tween 80 hydrolysis	Positive	Negative[2]
Urease	Positive	Negative[2]
Growth in the presence of 5% sodium chloride	Negative	Weak Positive[2]
Growth in the presence of thiophene-2-carboxylic acid hydrazide (TCH)	Positive	Positive
Genotypic Analysis		
Sequencing of 16S ribosomal RNA gene (~ 900 base pairs)	≥ 99% sequence identity to *M. avium* type strain (GenBank: CP046507.1)	100% sequence identity to *M. avium* type strain (GenBank: CP046507.1)[4]
Sequencing of Heat Shock Protein 65 gene (~ 440 base pairs)	≥ 99% sequence identity to *M. avium* type strain (GenBank: CP046507.1)	100% sequence identity to *M. avium* type strain (GenBank: CP046507.1)
Digital DNA-DNA hybridization (dDDH)[5]	≥ 70% for species identification	*M. avium* (93.2%)[6] *M. avium* subsp. *silvaticum* (92.7%) *M. bouchedurhonense* (91.6%)[7]

TEST	SPECIFICATIONS	RESULTS
Purity (post-freeze)		
Middlebrook 7H10 agar with OADC enrichment[8] 14 days at 37°C in an aerobic atmosphere with 5% CO_2	Growth consistent with expected colony morphology	Growth consistent with expected colony morphology
Tryptic Soy agar with 5% defibrinated sheep blood 14 days at 37°C in an aerobic atmosphere with 5% CO_2	Report results	Growth consistent with expected colony morphology
Viability	Growth	Growth

[1] Information on *Mycobacterium* testing is available from Ribón, W. "Biochemical Isolation and Identification of Mycobacteria." Biochemical Testing. (2012) Jose C. Jimenez-Lopez (Ed.), InTech, Available from: Biochemical Isolation and Identification of Mycobacteria, Lévy-Frébault, V. V. and F. Portaels. "Proposed Minimal Standards for the Genus *Mycobacterium* and for Description of New Slowly Growing *Mycobacterium* Species." Int. J. Syst. Bacteriol. 42 (1992): 315-323. PubMed: 1581193, and Magee, J. G. and A. C. Ward. "Family III. *Mycobacteriaceae* Chester 1897, 63^{AL}." Bergey's® Manual of Systematic Bacteriology, Volume 5. (2012) Goodfellow, M., et al. (Ed.), Springer.

[2] The specification for this test was established using the test result from BEI Resources seed lot 62009757. Species-level identification and characterization of *Mycobacterium* spp. through biochemical testing has been shown to produce inconsistent or nonreproducible results. The use of whole genome sequencing and digital DNA-DNA hybridization (dDDH) provides quantitative species-level identification that is accurate, precise, and reproducible. BEI Resources will be discontinuing the use of biochemical testing to identify *Mycobacterium* spp. in favor of WGS/dDDH. For more information, refer to Forbes, B. A., et al. "Practical Guidance for Clinical Microbiology Laboratories: Mycobacteria." Clin. Microbiol. Rev. 31 (2018): e00038-17. PubMed: 29386234.

[3] Negative tests are observed for > 7 days.

[4] Also consistent with other members of the *M. avium* complex

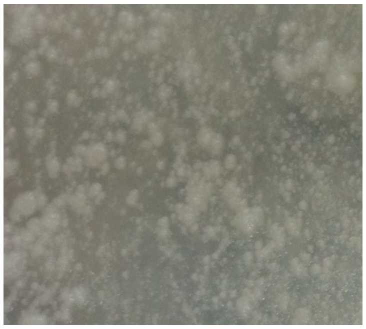
[5] Relatedness between bacterial strains has traditionally been determined using DDH. For additional information refer to Auch, A.F., et al. "Digital DNA_DNA Hybridization for Microbial Species Delineation by Means of Genome-to-Genome Sequence Comparison." Stand. Genomic Sci. 2 (2010): 117-134. PubMed: 21304684.

[6] The whole genome of *M. avium* subsp. *avium*, strain 2285 Smooth (contig total length approximately 5.19 megabase pairs) was sequenced using the Illumina® NextSeq® system.

[7] *M. bouchedurhonense* is a member of the *M. avium* species complex (Ben Salah, I., et al. "*Mycobacterium marseillense* sp. nov., *Mycobacterium timonense* sp. nov. and *Mycobacterium bouchedurhonense* sp. nov., Members of the *Mycobacterium avium* Complex." Int. J. Sys. Evol. Microbiol. 59 (2009): 2803-2808. PubMed: 19628609.).

[8] M7H10 agar with OADC enrichment contains malachite green, which may inhibit the growth of contaminating microorganisms.

Figure 1: Colony Morphology



/Sonia Bjorum Brower/
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