b|e|i RESOURCES
SUPPORTING INFECTIOUS DISEASE RESEARCH

Certificate of Analysis for NR-49075

Mycobacterium monacense, Strain B9-21-178T

Catalog No. NR-49075

Product Description: *Mycobacterium monacense (M. monacense)*, strain B9-21-178T was isolated in 2008 from bronchial lavage of an 80-year-old patient with chronic multifocal lung carcinoma and insulin-dependent diabetes mellitus in Munich, Germany.

Lot[1]: 64362402 **Manufacturing Date: 08JUL2016**

TEST	SPECIFICATIONS	RESULTS
Phenotypic Analysis[2,3]		
Cellular morphology	Report results	Rods
Colony morphology[4]	Report results	Circular, flat, undulate, rough and cream (Figure 1)
Growth on MacConkey agar (without crystal violet)	Negative	Negative
Growth rate	≤ 7 days	4 days
Growth at 45°C	Positive	Positive
Growth at 55°C	Report results	Negative
Acid-fast stain	Positive (red colonies)	Positive (red colonies)
Biochemical tests		
Nitrate reduction	Positive	Positive
Aryl sulfate (3 days)	Negative	Negative
Aryl sulfate (14 days)	Report results	Positive
Iron uptake	Report results	Positive
Growth in the presence of 5% sodium chloride	Positive	Positive
Growth in the presence of thiophene-2-carboxylic acid hydrazide (TCH)	Report results	Positive
Genotypic Analysis		
Sequencing of 16S ribosomal RNA gene (~ 1470 base pairs)	≥ 99% sequence identity to *M. monacense* type strain (GenBank: AF107039.2)	100% sequence identity to *M. monacense* type strain (GenBank: AF107039.2)
Digital DNA-DNA hybridization (dDDH)[5]	≥ 70% for species identification	*M. monacense* (96.4%)[6]
Purity (post-freeze)		
Middlebrook 7H10 agar with OADC enrichment[7]	Growth consistent with expected colony morphology	Growth consistent with expected colony morphology
Tryptic Soy agar[7]	Report results	Growth consistent with expected colony morphology
Viability (post-freeze)[4]	Growth	Growth

[1]NR-49075 was produced by inoculation of the deposited material in Middlebrook 7H9 broth with ADC enrichment for 4 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 3 days at 37°C in an aerobic atmosphere with 5% CO_2 to produce this lot.

[2]Information on Mycobacterium testing is available from Ribón, W. "Biochemical Isolation and Identification of Mycobacteria." Biochemical Testing. (2012) Jose C. Jimenez-Lopez (Ed.), InTech, http://www.intechopen.com/books/biochemical-testing/biochemical-isolation-and-identification-of-mycobacteria and 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.

[3]Phenotypic characterization of *M. monacense* was performed following: Reischl, U., et al. "*Mycobacterium monacense* sp. nov." Int. J. Syst. Evol. Microbiol. 56 (2006): 2575-2578. PubMed: 17082393.

[4]4 days at 37°C in an aerobic atmosphere with 5% CO_2 on Middlebrook 7H10 agar with OADC enrichment

[5]Relatedness between bacterial strains has traditionally been determined using dDDH. 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. monacense,* strain B9-21-178T (~ 6.1 megabase pairs) was sequenced using the Illumina® MiSeq® system and was assembled and analyzed with CLC Genomics Workbench Version 7.0.2.

[7]Purity of this lot was assessed for 8 days at 37°C in an aerobic atmosphere with 5% CO_2.

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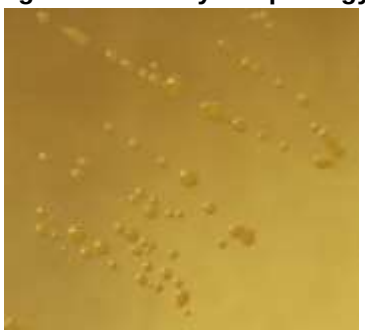
Figure 1: Colony Morphology



/Heather Couch/
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