Certificate of Analysis for NR-49074

Mycobacterium colombiense, Strain 10BT

Catalog No. NR-49074

Product Description: *Mycobacterium colombiense (M. colombiense)*, strain 10BT was isolated in 1995 from blood of an HIV-positive patient in Bogota, Colombia.

Lot[1]: 64362409 **Manufacturing Date: 29JUL2016**

TEST	SPECIFICATIONS	RESULTS
Phenotypic Analysis[2,3]		
Cellular morphology	Rods	Rods
Colony morphology[4]	Report results	Circular, convex, entire, smooth and white (Figure 1)
Growth rate	≥ 7 days	11 days
Growth at 45°C	Negative	Negative
Growth at 55°C	Report results	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 (no pigment)	Negative
Nonchromogen (no pigment)	Positive	Positive
Biochemical tests		
Catalase	Positive	Positive
Catalase (semiquantitative)	Report results	Positive
Catalase (68°C)	Report results	Positive
Iron uptake	Report results	Negative
Nitrate reduction	Report results	Negative
Tween 80 hydrolysis	Negative	Negative
Urease	Positive	Positive
Growth in the presence of 5% sodium chloride	Negative	Negative
Growth in the presence of thiophene-2-carboxylic acid hydrazide (TCH)	Report results	Positive
Genotypic Analysis		
Sequencing of 16S ribosomal RNA gene (~ 1800 base pairs)	≥ 99% sequence identity to *M. colombiense* type strain (GenBank: AM062764.1)	99.8% sequence identity to *M. colombiense* type strain (GenBank: AM062764.1)
Digital DNA-DNA hybridization (dDDH)[5]	≥ 70% for species identification	*M. colombiense* (96.7%)[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-49074 was produced by inoculation of the deposited material into Middlebrook 7H9 broth with ADC enrichment and grown for 11 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 7 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. colombiense* was performed following: Tortoli, E., et al. "*Mycobacterium colombiense* sp. nov., a Novel Member of the *Mycobacterium avium* Complex and Description of MAC-X as a New ITS Genetic Variant." Int. J. Syst. Evol. Microbiol. 56 (2006): 2049-2054. PubMed: 16957098.

[4]11 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 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.

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[6]The whole genome of *M. colombiense*, strain 10BT (Contig Total Length ~ 5.7 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 11 days at 37°C in an aerobic atmosphere with 5% CO_2.

Figure 1: Colony Morphology

Date: 21 DEC 2017 **Signature:**

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