Certificate of Analysis for NR-49072

Mycobacterium chimaera, Strain FI-01069T

Catalog No. NR-49072

Product Description:

Mycobacterium chimaera (M. chimaera), strain FI-01069T was isolated between 1999-2003 from sputum of a 56-year old female with bronchiectasis in Italy.

Lot: 70004908[1] **Manufacturing Date: 28JUN2017**

TEST	SPECIFICATIONS	RESULTS
Phenotypic Analysis[2]		
Cellular morphology	Rods	Rods
Colony morphology[3]	Report results	Circular, convex, entire, smooth and cream (Figure 1)
Growth rate	≥ 7 days	12 days
Growth at 45°C	Negative	Variable[4]
Growth at 55°C	Report results	Positive
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 (no pigment)
Nonchromogen (no pigment)	Positive (no pigment)	Positive (no pigment)
Biochemical tests		
Catalase	Report results	Negative
Catalase (semiquantitative)	Negative	Negative
Catalase (68°C)	Positive	Positive
Iron uptake	Report results	Negative
Nitrate reduction	Negative	Negative
Tween 80 hydrolysis	Negative	Negative
Urease	Negative	Negative
Growth in the presence of 5% sodium chloride	Negative	Positive[5]
Growth in the presence of thiophene-2-carboxylic acid hydrazide (TCH)	Positive	Positive
Genotypic Analysis		
Sequencing of Heat Shock Protein 65 gene (~ 700 base pairs)	≥ 99% sequence identity to *M. chimaera*, strain FI-0169T (GenBank: MRBR01000001.1)	100% sequence identity to *M. chimaera*, strain FI-0169T (GenBank: MRBR01000001.1)
Digital DNA-DNA hybridization (dDDH)[6]	≥ 70% for species identification	*M. chimaera* (99.7%)[7]
Purity (post-freeze)		
Middlebrook 7H10 agar with OADC enrichment[8]	Growth consistent with expected colony morphology	Growth consistent with expected colony morphology
Tryptic Soy agar[8]	Report results	Growth consistent with expected colony morphology
Viability (post-freeze)[3]	Growth	Growth

[1]NR-49072 was produced by inoculation of the deposited material into Middlebrook 7H9 broth with ADC enrichment and grown for 13 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 8 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]12 days at 37°C in an aerobic atmosphere with 5% CO_2 on Middlebrook 7H10 agar with OADC enrichment

[4]NR-49072 was deposited as *M. chimaera* and is reported to be negative for growth at 45°C. Testing performed by BEI Resources indicates growth was observed after 21 days at 45°C in an aerobic atmosphere in Middlebrook 7H9 broth with ADC enrichment. Growth was not observed after 21 days at 45°C in an aerobic atmosphere on Middlebrook 7H10 agar with OADC enrichment.

[5]NR-49072 was deposited as *M. chimaera* and is reported to be negative for growth in presence of 5% sodium chloride. Testing performed in triplicate by BEI Resources indicates a positive result.

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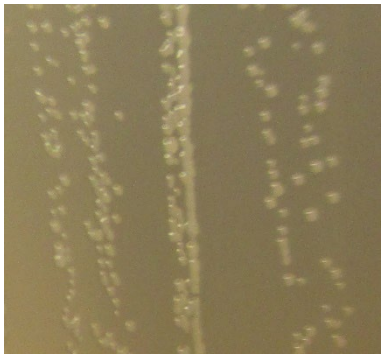
[6]Relatedness between bacterial strains has traditionally been determined using DDH. For additional information, refer to Auch, A. F., et al. "Digital DNA-DNA Hybridization for Microbial Species Delineation by Means of Genome-to-Genome Sequence Comparison." Stand. Genomic Sci. 2 (2010): 117-134. PubMed: 21304684.
[7]The whole genome of *M. chimaera*, strain FI-01069T (Contig Total length ~ 6.1 megabase pairs) was sequenced using the Illumina® MiSeq® system and was assembled and analyzed with CLC Genomics Workbench Version 7.0.2.
[8]Purity of this lot was assessed for 12 days at 37°C in an aerobic atmosphere with 5% CO_2.

Figure 1: Colony Morphology



/Heather Couch/
Heather Couch 24 SEP 2019
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