Certificate of Analysis for NR-49258

Mycobacterium caprae, Strain NLA000601960

Catalog No. NR-49258

Product Description:

Mycobacterium caprae (M. caprae), strain NLA000601960 was isolated in 2006 from human sputum in the Netherlands. NR-49258 was produced by inoculation of the deposited material into Middlebrook 7H9 broth with ADC enrichment. Broth inoculum was added to Middlebrook 7H10 agar with OADC enrichment kolles, which were grown for 29 days at 37°C in an aerobic atmosphere with 5% CO2 to produce this lot.

Lot: 70003656 **Manufacturing Date: 23JUN2017**

TEST	SPECIFICATIONS	RESULTS
Phenotypic Analysis[1,2]		
Cellular Morphology 21 days at 37°C in an aerobic atmosphere with 5% CO_2 on Middlebrook 7H10 agar with OADC enrichment	Report results	Gram-positive rods
Colony morphology	Report results	Irregular, slight peaked, undulate, rough and cream (Figure 1)
Growth rate	≥ 7 days	21 days
Growth at 26°C	Negative	Negative
Growth at 37°C	Positive	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	Negative
Nonchromogen (no pigment)	Positive	Positive
Biochemical tests		
Niacin production[3]	Negative	Negative
Nitrate reduction	Negative	Negative
Pyrazinamidase	Positive	Positive
Genotypic Analysis		
Sequencing of Heat Shock Protein 65 gene (~ 420 base pairs)	≥ 99% sequence identity to *M. caprae* type strain (GenBank: AF547884.1)	100% sequence identity to *M. caprae* type strain (GenBank: AF547884.1)[4]
Digital DNA-DNA hybridization (dDDH)[5]	≥ 70% for species identification	*M.* caprae (98.9%)[6,7] *M. tuberculosis* (97.9%)[8] *M. africanum* (98.6%)[8] *M. bovis* (98.3%)[8] *M. caprae* (98.9%)[8] *M. microti* (98.5%)[8] *M. mungi* (98.7%)[8] *M. orygis* (98.5%)[8] *M. pinnipedii* (98.0%)[8] *M. canettii* (92%)[8]
Spacer oligonucleotide typing	Report results	200003777377600 (BOV_4-CAPRAE)[9]
Purity (post-freeze)		
Middlebrook 7H10 agar with OADC enrichment 49 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 46 days at 37°C in an aerobic atmosphere with 5% CO_2	Report results	Growth consistent with expected colony morphology
Viability 21 days at 37°C in an aerobic atmosphere with 5% CO_2 on Middlebrook 7H10 agar with OADC enrichment	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, 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.

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[2]Phenotypic characterization of *M. caprae* was performed following: Aranaz, A., et al. "*Mycobacterium tuberculosis* subsp. *caprae* subsp. nov.: A Taxonomic Study of a New Member of the *Mycobacterium tuberculosis* Complex Isolated from Goats in Spain." Int. J. Syst. Bacteriol. 49 (1999): 1263-1273. PubMed: 10425790.
[3]All mycobacteria produce niacin but only *M. tuberculosis* accumulates it, resulting in a positive test for *M. tuberculosis*.
[4]Also consistent with *M. africanum, M. bovis, M. canettii, M. microti* and *M. tuberculosis*
[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. caprae,* strain NLA0000601960 (~ 4.3 megabase pairs) was sequenced using the Illumina® MiSeq® system and was assembled and analyzed using PATRIC Comprehensive Genome Analysis with the SPAdes pipeline.
[7]The required whole genome sequence for the type strain of this variant is not available. *M. tuberculosis* variant caprae, strain ATCC BAA-824 (GenBank: MWXD01000000) was used for dDDH analysis.
[8]Species within the *Mycobacterium tuberculosis* complex cannot be differentiated by DNA-DNA hybridization due to 90-100% DNA relatedness between the individual species (Imaeda, T. "Deoxyribonucleic Acid Relatedness Among Selected Strains of *Mycobacterium tuberculosis, Mycobacterium bovis, Mycobacterium bovis* BCG, *Mycobacterium microti,* and *Mycobacterium africanum.*" Int. J. Syst. Bacteriol. 35 (1985): 147-150.).
[9]Spacer oligonucleotide typing (spoligotyping) is a widely used genotyping method for *Mycobacterium tuberculosis* variants in which a set of 43 unique spacer sequences is translated into a 15-digit code that is used to classify strains (Xia, E., Y. Y. Teo and R. T. Ong. "SpoTyping: Fast and Accurate *in silico Mycobacterium* Spoligotyping from Sequence Reads." Genome Med. 8 (2016):19. PubMed: 26883915.).

Figure 1: Colony Morphology

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