Certificate of Analysis for NR-49254

Mycobacterium microti, Strain NLA000015496

Catalog No. NR-49254

Product Description: *Mycobacterium microti* (*M. microti*), strain NLA000015496 was isolated from a vole in the United Kingdom.

Lot[1]: 63954348 **Manufacturing Date: 18MAR2016**

TEST	SPECIFICATIONS	RESULTS
Phenotypic Analysis[2,3]		
Cellular morphology	Gram-positive rods	Gram-positive rods
Colony morphology[4]	Report results	Irregular, slight peaked, undulate, rough and cream (Figure 1)
Growth rate	≥ 7 days	40 days
Growth at 26°C	Report results	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 (no pigment)	Negative (no pigment)
Nonchromogen (no pigment)	Positive (no pigment)	Positive (no pigment)
Biochemical tests		
Niacin production[5]	Report results	Positive
Nitrate reduction	Report results	Negative
Pyrazinamidase	Report results	Positive
Genotypic Analysis		
Sequencing of Heat Shock Protein 65 gene (~ 390 base pairs)	≥ 99% sequence identity to *M. microti* type strain (GenBank: AY299135.1)	100% sequence identity to *M. microti* type strain (GenBank: AY299135.1)[6]
Digital DNA-DNA hybridization (dDDH)[7]	≥ 70% for species identification	*M. microti* (99.2%)[8,9] *M. pinnipedii* (98.7%)[9] *M. africanum* (98.6%)[9] *M. caprae* (98.5%)[9] *M. tuberculosis* (98.3%)[9] *M. bovis* (97.9%)[9]
Purity (post-freeze)		
Middlebrook 7H10 agar with OADC enrichment[10]	Growth consistent with expected colony morphology	Growth consistent with expected colony morphology
Tryptic Soy agar[10]	Report results	No growth
Viability (post-freeze)[4]	Growth	Growth

[1]NR-49254 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 37 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. microti* was performed following: 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, van Soolingen, D., et al. "Diagnosis of *Mycobacterium microti* Infections Among Humans by Using Novel Genetic Markers." J. Clin. Microbiol. 36 (1998): 1840-1845. PubMed: 9650922.

[4]The colony morphology was observed after 40 days at 37°C in an aerobic atmosphere with 5% CO_2 on Middlebrook 7H10 agar with OADC enrichment.

[5]While a positive niacin result has traditionally been used to differentiate *M. tuberculosis* from other *Mycobacteria,* both positive and negative niacin results for *M. microti* have been reported in the literature.

[6]Also consistent with *M. africanum, M. bovis, M. caprae, M. canettii* and *M. tuberculosis*

[7]Relatedness between bacterial strains has traditionally been determined using dDDH. For additional information refer to Auch, A.F., et al. "Digital

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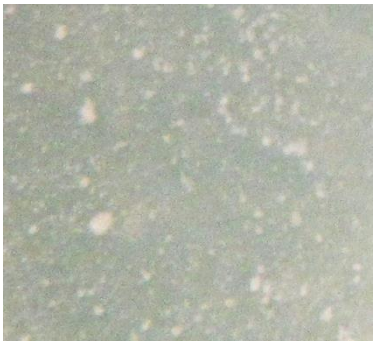
DNA-DNA Hybridization for Microbial Species Delineation by Means of Genome-to-Genome Sequence Comparison." Stand Genomic Sci. 2 (2010): 117-134. PubMed: 21304684.

[8]The whole genome of *M. microti,* strain NLA000015496 was sequenced using the Illumina® MiSeq® system and was assembled and analyzed with CLC Genomics Workbench Version 7.0.2.

[9]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.).

[10]Purity of this lot was assessed for 41 days at 37°C in an aerobic atmosphere with 5% CO_2.

Figure 1: Colony Morphology



Date: 16 OCT 2017

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