Certificate of Analysis for NR-44264

Mycobacterium avium, Strain 2285 Rough

Catalog No. NR-44264

Product Description: *Mycobacterium avium* (*M. avium*), strain 2285 Rough was isolated between 2009 and 2013 from human sputum at the National Institutes for Allergy and Infectious Diseases (NIAID), National Institutes of Health (NIH), Bethesda, Maryland, USA.

Lot[1]: 62009759 **Manufacturing Date: 08NOV2013**

TEST	SPECIFICATIONS	RESULTS
Phenotypic Analysis[2,3]		
Cellular morphology	Gram-positive rods	Gram-positive rods
Colony morphology[4]	Report results	Irregular, low convex, undulate, rough, opaque and cream (Figure 1)
Motility (wet mount)	Report results	Non-motile
Growth on Brain Heart Infusion agar	Report results	Growth
Growth rate	≥ 7 days	≥ 7 days
Growth at 26°C	Report results	Positive
Growth at 37°C	Positive	Positive
Growth at 45°C	Report results	Positive
Growth at 55°C	Report results	Negative
Acid-fast stain	Positive (red colonies)	Positive (red colonies)
Pigmentation	Nonchromogen	Scotochromogen[5]
Biochemical tests[6]		
Nitrate reduction	Negative	Negative
Pyrazinamidase	Report results	Positive
Urease	Negative	Positive[7]
Catalase	Positive	Positive
Semiquantitative catalase	Report results	Negative
Heat-stable catalase	Report results	Positive
Iron uptake	Negative	Negative
Tween 80 hydrolysis	Negative	Negative
Growth in the presence of 5% sodium chloride	Negative	Positive[7]
Growth in the presence of thiophene-2-carboxylic acid hydrazide (TCH)	Positive	Positive
Genotypic Analysis[8]		
Whole Genome Sequencing (~ 5.2 megabase pairs)	Report results	Consistent with *M. avium*
Purity (post-freeze)[9,10]	Growth consistent with expected colony morphology	Growth consistent with expected colony morphology
Viability (post-freeze)[4]	Growth	Growth

[1]NR-44264 was produced by inoculation of the deposited material in Middlebrook 7H9 broth with ADC enrichment for 19 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 18 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, and Magee, J. G. and A. C. Ward. "Family III. *Mycobacteriaceae* Chester 1897, 63^{AL}." Bergey's® Manual of Systematic Bacteriology, Second Edition, Volume Five. (2012) Goodfellow, M., et al. (Ed.), Springer.

[3]Phenotypic tests rule out other slow-growing *Mycobacterium* species [Magee, J. G. and A. C. Ward. "Family III. *Mycobacteriaceae* Chester 1897, 63^{AL}." Bergey's® Manual of Systematic Bacteriology, Second Edition, Volume Five. (2012) Goodfellow, M., et al. (Ed.), Springer].

[4]18 days at 37°C in an aerobic atmosphere with 5% CO_2 on Middlebrook 7H10 Agar with OADC enrichment

[5]Specification for this test was obtained from 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, which

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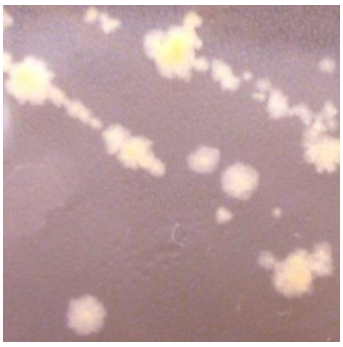
indicates that most strains of *M. avium* are nonchromogens and show no pigment; however, a few strains (~ 15%) may develop pigmentation as they age.
[6]Negative tests are observed for > 7 days.
[7]Specifications for these tests were obtained from Lévy-Frébault, V. V. and F. Portaels. "Proposed Minimal Standards for the Genus *Mycobacterium* and for Description of New Slowly Growing *Mycobacterium* Species." Int. J. Syst. Bacteriol. 42 (1992): 315-323. PubMed: 1581193, which indicates that most strains of *M. avium* are negative for this test; however up to 15% of strains may be positive.
[8]Illumina® MiSeq® sequence was analyzed with CLC Genomics Workbench Version 7.0.2.
[9]Purity of this lot was assessed for 18 days at 37°C in an aerobic atmosphere with 5% CO_2 on Middlebrook 7H10 agar with OADC enrichment.
[10]Middlebrook 7H10 agar with OADC enrichment contains malachite green, which may inhibit growth of contaminating microorganisms.

Figure 1: Colony Morphology



Date: 04 NOV 2015 **Signature:**

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