b|e|i RESOURCES
SUPPORTING INFECTIOUS DISEASE RESEARCH

Certificate of Analysis for NR-49085

Mycobacterium celeriflavum, Strain AFPC000207T

Catalog No. NR-49085

Product Description: *Mycobacterium celeriflavum (M. celeriflavum)*, strain AFPC000207T was isolated in 2010 from the sputum of a 44-year-old male patient with chronic obstructive pulmonary disease in Ahvaz, Iran.

Lot[1]: 64362397 **Manufacturing Date: 11JUL2016**

TEST	SPECIFICATIONS	RESULTS
Phenotypic Analysis[2,3]		
Cellular morphology	Report results	Rods
Colony morphology[4]	Report results	Circular, convex, entire, rough and cream
Growth on MacConkey agar (without crystal violet)	Negative	Negative
Growth rate	≤ 7 days	4 days
Growth at 45°C	Negative	Positive[5]
Growth at 55°C	Report results	Positive
Acid-fast stain	Positive (red colonies)	Positive (red colonies)
Biochemical tests		
Nitrate reduction	Positive	Negative[6]
Aryl sulfate (3 days)	Report results	Negative
Aryl sulfate (14 days)	Report results	Positive
Iron uptake	Report results	Negative
Growth in the presence of 5% sodium chloride	Report results	Positive
Growth in the presence of thiophene-2-carboxylic acid hydrazide (TCH)	Report results	Positive
Genotypic Analysis		
Sequencing of 16S ribosomal RNA gene (~ 1440 base pairs)	≥ 99% sequence identity to *M. celeriflavum* type strain (GenBank: KJ607136.1)	100% sequence identity to *M. celeriflavum* type strain (GenBank: KJ607136.1)
Digital DNA-DNA hybridization (dDDH)[7]	≥ 70% for species identification	*M. celeriflavum* (99.7%)[8]
Purity (post-freeze)		
Middlebrook 7H10 agar with OADC enrichment[9]	Growth consistent with expected colony morphology	Growth consistent with expected colony morphology
Tryptic Soy agar[9]	Report results	Growth consistent with expected colony morphology
Viability (post-freeze)[4]	Growth	Growth

[1]NR-49085 was produced by inoculation of the deposited material in Middlebrook 7H9 broth with ADC enrichment for 5 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 5 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. celeriflavum* was performed following: Shahraki, A. H., et al. "*Mycobacterium celeriflavum* sp. nov., a Rapidly Growing Scotochromogenic Bacterium isolated from Clinical Specimens." Int. J. Syst. Evol. Microbiol. 65 (2015): 510-515. PubMed: 25389151.

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

[5]NR-49085 was deposited as *M. celeriflavum* and reported to be negative for growth at 42°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 and after 7 days at 45°C in an aerobic atmosphere on Middlebrook 7H10 agar with OADC enrichment and Lowenstein-Jensen agar.

[6]NR-49085 was deposited as *M. celeriflavum* and reported to be positive for nitrate reduction. Testing performed by BEI Resources indicates a negative result.

[7]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.

BEI Resources
www.beiresources.org
E-mail: contact@beiresources.org
Tel: 800-359-7370
Fax: 703-365-2898

[8]The whole genome of *M. celeriflavum,* strain AFPC000207T (Contig Total Length ~ 4.98 megabase pairs) was sequenced using the Illumina® MiSeq® system and was assembled and analyzed with CLC Genomics Workbench Version 7.0.2.
[9]Purity of this lot was assessed for 8 days at 37°C in an aerobic atmosphere with 5% CO_2.

/Heather Couch/
Heather Couch 09 JUL 2018
Program Manager or designee, ATCC Federal Solutions

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