

## Certificate of Analysis for NR-49069

## Mycobacterium conspicuum, Strain FI-95138

Catalog No. NR-49069

Product Description: Mycobacterium conspicuum (M. conspicuum), strain FI-95138 was isolated

from sputum.

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Lot<sup>1</sup>: 64362410 Manufacturing Date: 04AUG2016

TEST	SPECIFICATIONS	RESULTS	
Phenotypic Analysis <sup>2,3</sup>			
Cellular morphology	Rods	Rods	
Colony morphology <sup>4</sup>	Report results	Circular, low convex, entire, smooth	
Colony morphology	Teport results	and cream (Figure 1)	
Growth on MacConkey agar (without crystal violet)	No growth	No growth	
Growth rate	≥ 7 days	17 days	
Growth at 45°C	Report results	Variable <sup>5</sup>	
Growth at 55°C	Report results	Negative	
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	Positive	Positive	
Catalase (semiquantitative)	Report results	Negative	
Catalase (68°C)	Positive	Positive	
Iron uptake	Report results	Negative	
Nitrate reduction	Negative	Negative	
Tween 80 hydrolysis	Positive	Positive	
Urease	Negative	Negative	
Growth in the presence of 5% sodium chloride	Negative	Negative	
Growth in the presence of thiophene-2-carboxylic acid	Positive	Negative <sup>6</sup>	
hydrazide (TCH)			
Genotypic Analysis			
Sequencing of 16S ribosomal RNA gene	≥ 99% sequence identity to	100% sequence identity to	
(~ 1430 base pairs)	M. conspicuum type strain	M. conspicuum type strain	
,	(GenBank: X88922.1)	(GenBank: X88922.1)	
Digital DNA-DNA hybridization (dDDH) <sup>7</sup>	≥ 70% for species identification	Not determined <sup>8,9</sup> (Table 1)	
Purity (post-freeze)			
Middlebrook 7H10 agar with OADC enrichment <sup>10</sup>	Growth consistent with expected	Growth consistent with expected	
	colony morphology	colony morphology	
Tryptic Soy agar <sup>10</sup>	Report results	Growth consistent with expected	
		colony morphology	
Viability (post-freeze) <sup>4</sup>	Growth	Growth	

<sup>&</sup>lt;sup>1</sup>NR-49069 was produced by inoculation of the deposited material into Middlebrook 7H9 broth with ADC enrichment and grown for 15 days at 37°C in an aerobic atmosphere with 5% CO<sub>2</sub>. Broth inoculum was added to Middlebrook 7H10 agar with OADC enrichment kolles, which were grown for 9 days at 37°C in an aerobic atmosphere with 5% CO<sub>2</sub> to produce this lot.

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<sup>&</sup>lt;sup>2</sup>Information on *Mycobacterium* testing is available from Ribón, W. "Biochemical Isolation and Identification of Mycobacteria." <u>Biochemical Testing.</u> (2012) Jose C. Jimenez-Lopez (Ed.), InTech, <a href="http://www.intechopen.com/books/biochemical-testing/biochemical-isolation-and-identification-of-mycobacteria">http://www.intechopen.com/books/biochemical-testing/biochemical-isolation-and-identification-of-mycobacteria</a> and Lévy-Frébault, V. V. and F. Portaels. "Proposed Minimal Standards for the Genus *Mycobacterium* and for Description of New Slowly Growing *Mycobacterium* Species." <u>Int. J. Syst. Bacteriol.</u> 42 (1992): 315-323. PubMed: 1581193.

<sup>&</sup>lt;sup>3</sup>Phenotypic characterization of *M. conspicuum* was performed following: Spring, B., et al. "*M. conspicuum* sp. nov., a New Species Isolated from Patients with Disseminated Infections." <u>J. Clin. Microbiol.</u> 33 (1995): 2805-2811. PubMed: 8576323.

<sup>&</sup>lt;sup>4</sup>17 days at 37°C in an aerobic atmosphere with 5% CO<sub>2</sub> on Middlebrook 7H10 agar with OADC enrichment

<sup>&</sup>lt;sup>5</sup>NR-49069 was deposited as *M. conspicuum* and reported to be negative for growth at 41°C. Testing performed by BEI Resources indicates growth was observed after 7 days at 45°C in an aerobic atmosphere in Middlebrook 7H9 broth with ADC enrichment. Growth was not observed after 14 days at 45°C in an aerobic atmosphere on Lowenstein-Jensen agar.



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Figure 1: Colony Morphology

Figure 2: Digital DNA-DNA hybridization (dDDH)

Species	Strain	Accession #	GGD vs. NR-49069 (Deposited as: <i>M. conspicuum</i> )
M. abscessus subsp. abscessus	Hauduroy L948 <sup>™</sup>	NC_010397.1	19.3
M. abscessus subsp. bolletii	$BD^T$	AHAS00000000.1	19.5
M. abscessus subsp. massiliense	CCUG 48898 <sup>™</sup>	NZ_AP014547.1	19.6
M. aromaticivorans	JS19b1 <sup>⊤</sup>	JALN00000000.2	20.1
M. aurum	ATCC 23366 <sup>T</sup>	CVQQ01000001.1	20.1
M. austroafricanum	E9789-SA12441 <sup>T</sup>	HG964450.1	19.7
M. avium subsp. avium	ATCC 25291 <sup>™</sup>	ACFI00000000.1	23.9
M. avium subsp. paratuberculosis	ATCC 19698 <sup>T</sup>	AGAR00000000.1	24.5
M. avium subsp. silvaticum	6409 <sup>T</sup>	AYOC00000000.1	24.3
M. bohemicum	CIP 105808 <sup>T</sup>	CSTD01000001.1	23.6
M. canariasense	502329 <sup>T</sup>	BCSY00000000.1	20.2
M. celatum	ATCC 51131 <sup>™</sup>	BBUN00000000.1	22.4
M. chelonae	CM 6388 <sup>T</sup>	CP010946.1	19.7
M. chlorophenicolum	PCP-I <sup>™</sup>	JYNL00000000.1	20.2
M. chubuense	48013 <sup>™</sup>	NC_018027.1	20.2
M. colombiense	10B <sup>T</sup>	AFVW00000000.2	23.5
M. conceptionense	D16 <sup>T</sup>	CTEF00000000.1	20.2
M. cosmeticum	LTA-388 <sup>™</sup>	CCBB000000000.1	20
M. crocinum	czh-42 <sup>™</sup>	BBHD00000000.1	21.9
M. farcinogenes	IEMVT 75 <sup>™</sup>	CCAY000000000.1	20.1
M. fluoranthenivorans	FA4 <sup>T</sup>	BBFT00000000.1	21.2
M. fortuitum subsp. fortuitum	ATCC 6841 <sup>T</sup>	CP014258.1	20
M. fortuitum subsp. acetamidolyticum	NCH E11620 <sup>T</sup>	BCSZ00000000.1	20
M. gastri	ATCC 15754 <sup>T</sup>	AZYN00000000.1	23.1
M. genavense	2289 <sup>T</sup>	JAGZ00000000.1	23.5

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<sup>&</sup>lt;sup>6</sup>NR-49069 was deposited as *M. conspicuum* and reported to be positive for growth in the presence of TCH. Testing performed by BEI Resources indicates a negative result.

<sup>&</sup>lt;sup>7</sup>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." <u>Stand Genomic Sci.</u> 2 (2010): 117-134, PubMed: 21304684.

<sup>&</sup>lt;sup>8</sup>The whole genome of *M. conspicuum*, strain FI-95138 (Contig Total Length ~ 5.9 megabase pairs) was sequenced using the Illumina<sup>®</sup> MiSeq<sup>®</sup> system and was assembled and analyzed with CLC Genomics Workbench Version 7.0.2.

<sup>&</sup>lt;sup>9</sup>The required whole genome sequence for the type strain of this species is not available. dDDH testing rules out all species listed in Figure 2, however, this does not rule out species for which the type strains whole genome sequences are not available.

<sup>&</sup>lt;sup>10</sup>Purity of this lot was assessed for 17 days at 37°C in an aerobic atmosphere with 5% CO<sub>2</sub>.



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Species	Strain	Accession #	GGD vs. NR-49069 (Deposited as: <i>M. conspicuum</i> )
M. haemophilum	ATCC 29548 <sup>T</sup>	CP011883.2	23.1
M. hassiacum	3849 <sup>T</sup>	ARBU00000000.1	20.6
M. hodleri	EMI2 <sup>⊤</sup>	BBGO00000000.1	23.1
M. intracellulare	ATCC 13950 <sup>T</sup>	NC_016946.1	23.6
M. kansasii	ATCC 12478 <sup>T</sup>	NC_022663.1	22.6
M. kyorinense	KUM 060204 <sup>T</sup>	BBKA00000000.1	21.8
M. mageritense	938 <sup>T</sup>	CCBF000000000.1	20.1
M. neoaurum	ATCC 25795 <sup>™</sup>	JMDW00000000.1	19.6
M. neworleansense	W6705 <sup>™</sup>	CWKH00000000.1	19.8
M. novocastrense	73 <sup>T</sup>	BCTA00000000.1	20.3
M. obuense	47001 <sup>⊤</sup>	JYNU00000000.1	20.1
M. pallens	czh-8 <sup>⊤</sup>	BBHE00000000.1	21.9
M. parascrofulaceum	HSC-68 <sup>™</sup>	ADNV00000000.1	24
M. pseudoshottsii	L15 <sup>T</sup>	BCND00000000.1	21.7
M. pyrenivorans	17A3 <sup>T</sup>	BBHB00000000.1	22.2
M. rufum	JS14 <sup>™</sup>	JROA00000000.1	20.3
M. rutilum	czh-117 <sup>⊤</sup>	BBHF00000000.1	23.9
M. septicum	W4964 <sup>T</sup>	CBMO000000000.1	20.1
M. setense	ABO-M06 <sup>T</sup>	JTJW00000000.1	19.7
M. simiae	ATCC 25275 <sup>™</sup>	CBMJ0000000000.2	22.6
M. smegmatis	ATCC 19420 <sup>T</sup>	LN831039.1	20.4
M. thermoresistibile	ATCC 19527 <sup>™</sup>	BCTB00000000.1	20.6
M. triplex	90-1019 <sup>T</sup>	CCAU000000000.1	23.7
M. tuberculosis	H37Rv <sup>™</sup>	NC_000962.3	22.7
M. vaccae	ATCC 15483 <sup>T</sup>	BCRS00000000.1	20.3
M. vanbaalenii	PYR-1 <sup>⊤</sup>	NC_008726.1	20.3
M. vulneris	NLA000700772 <sup>T</sup>	CCBG000000000.1	20.1
M. yongonense	05-1390 <sup>T</sup>	NC_021715.1	23.5
Nocardia asteroides	NBRC 15531 <sup>™</sup>	BAFO00000000.2	19.6

**Date:** 24 JUL 2017

Signature:

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