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SUPPORTING INFECTIOUS DISEASE RESEARCH

Certificate of Analysis for NR-49087

Mycobacterium paraense, Strain IEC26T

Catalog No. NR-49087

Product Description: *Mycobacterium paraense* (*M. paraense*), strain IEC26T was isolated between 2009 and 2010 from the sputum of a patient in Parauapebas, Pará, Brazil.

Lot[1]: 64362429 **Manufacturing Date: 01AUG2016**

TEST	SPECIFICATIONS	RESULTS
Phenotypic Analysis[2,3]		
Cellular morphology	Gram-positive rods	Rods
Colony morphologies[4,5]	Report results	Colony type 1: Circular, convex, entire, cream and smooth (Figure 1) Colony type 2: Circular, flat, entire, opaque and cream (Figure 1)
Growth rate	≥ 7 days	13 days
Growth at 45°C	Negative	Variable[6]
Growth at 55°C	Report results	Negative
Acid-fast stain	Positive (red colonies)	Positive (red colonies)
Pigmentation in the dark (Scotochromogen)	Positive	Positive
Photoinduction for 1 hour (Photochromogen)	Negative	Negative
Nonchromogen (no pigment)	Negative	Negative
Biochemical tests		
Catalase	Positive	Positive
Catalase (semiquantitative)	Report results	Positive
Catalase (68°C)	Positive	Positive
Iron uptake	Report results	Negative
Nitrate reduction	Negative	Negative
Tween 80 hydrolysis	Report results	Positive
Urease	Negative	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	Negative
Genotypic Analysis		
Sequencing of 16S ribosomal RNA gene (1480 base pairs)	≥ 99% sequence identity to *M. paraense* type strain (GenBank: KJ948996.1)	100% sequence identity to *M. paraense* type strain (GenBank: KJ948996.1)
Digital DNA-DNA hybridization (dDDH)[7]	≥ 70% for species identification	Not determined[8,9] (Table 1)
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	Growth consistent with expected colony morphology
Viability (post-freeze)[4]	Growth	Growth

[1]NR-49087 was produced by inoculation of the deposited material into Middlebrook 7H9 broth with ADC enrichment and grown for 14 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 6 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. paraense* was performed following: Fusco da Costa, A. R., et al. "Characterization of 17 Strains Belonging to the *Mycobacterium simiae* Complex and Description of *Mycobacterium paraense* sp. nov." Int. J. Syst. Evol. Microbiol. 65 (2012): 656-662. PubMed: 25487637.

[4]13 days at 37°C in an aerobic atmosphere with 5% CO_2 on Middlebrook 7H10 agar with OADC enrichment

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[5]Two colony types were observed. The heat shock protein 65 gene of each colony type was sequenced and found to be consistent with the other colony type.
[6]NR-49087 was deposited as *M. paraense* and reported to be negative for growth at 42°C. Testing performed by BEI Resources indicates growth was observed after 14 days at 45°C in an aerobic atmosphere on Lowenstein-Jensen agar and in Middlebrook 7H9 broth with ADC enrichment and growth was not observed after 21 days at 45°C in an aerobic atmosphere on Middlebrook 7H10 agar with OADC enrichment.
[7]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." Stand. Genomic Sci. 2 (2010): 117-134. PubMed: 21304684.
[8]The whole genome of *M. paraense*, strain IEC26T (Contig Total Length ~ 5.6 megabase pairs) was sequenced using the Illumina® MiSeq® system and was assembled and analyzed with CLC Genomics Workbench Version 7.0.2.
[9]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.
[10]Purity of this lot was assessed for 13 days at 37°C in an aerobic atmosphere with 5% CO_2.

Figure 1: Colony Morphology

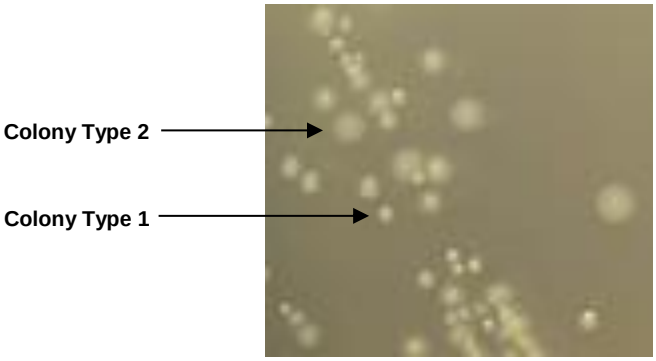

Figure 2: Digital DNA-DNA hybridization (dDDH)

Species	Strain	Accession #	GGD vs. NR-49087 (Deposited as: *M. paraense*)
M. abscessus subsp. *abscessus*	Hauduroy L948^T	NC_010397.1	19.6
M. abscessus subsp. *bolletii*	BD^T	AHAS00000000.1	19.2
M. abscessus subsp. *massiliense*	CCUG 48898^T	NZ_AP014547.1	19.4
M. aromaticivorans	JS19b1^T	JALN00000000.2	19.9
M. aurum	ATCC 23366^T	CVQQ01000001.1	20.5
M. austroafricanum	E9789-SA12441^T	HG964450.1	20
M. avium subsp. *avium*	ATCC 25291^T	ACFI00000000.1	26.8
M. avium subsp. *paratuberculosis*	ATCC 19698^T	AGAR00000000.1	27.4
M. avium subsp. *silvaticum*	6409^T	AYOC00000000.1	27.3
M. bohemicum	CIP 105808^T	CSTD01000001.1	25.2
M. canariasense	502329^T	BCSY00000000.1	20.4
M. celatum	ATCC 51131^T	BBUN00000000.1	22.9
M. chelonae	CM 6388^T	CP010946.1	19.5
M. chlorophenicolum	PCP-I^T	JYNL00000000.1	20.5
M. chubuense	48013^T	NC_018027.1	20.1
M. colombiense	10B^T	AFVW00000000.2	26.3
M. conceptionense	D16^T	CTEF00000000.1	20.4
M. cosmeticum	LTA-388^T	CCBB000000000.1	20.5
M. crocinum	czh-42^T	BBHD00000000.1	21.9
M. farcinogenes	IEMVT 75^T	CCAY000000000.1	20.2
M. fluoranthenivorans	FA4^T	BBFT00000000.1	21.6
M. fortuitum subsp. *fortuitum*	ATCC 6841^T	CP014258.1	20

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Species	Strain	Accession #	GGD vs. NR-49087 (Deposited as: *M. paraense*)
M. fortuitum subsp. *acetamidolyticum*	NCH E11620^T	BCSZ00000000.1	20
M. gastri	ATCC 15754^T	AZYN00000000.1	22.9
M. genavense	2289^T	JAGZ00000000.1	23.9
M. haemophilum	ATCC 29548^T	CP011883.2	22.4
M. hassiacum	3849^T	ARBU00000000.1	20.5
M. hodleri	EMI2^T	BBGO00000000.1	22.8
M. intracellulare	ATCC 13950^T	NC_016946.1	26.4
M. kansasii	ATCC 12478^T	NC_022663.1	22.6
M. kyorinense	KUM 060204^T	BBKA00000000.1	22.2
M. mageritense	938^T	CCBF000000000.1	20.2
M. neoaurum	ATCC 25795^T	JMDW00000000.1	19.9
M. novocastrense	73^T	BCTA00000000.1	20.4
M. obuense	47001^T	JYNU00000000.1	20.1
M. pallens	czh-8^T	BBHE00000000.1	21.8
M. parascrofulaceum	HSC-68^T	ADNV00000000.1	34.7
M. pseudoshottsii	L15^T	BCND00000000.1	21.7
M. pyrenivorans	17A3^T	BBHB00000000.1	22.5
M. rufum	JS14^T	JROA00000000.1	20.6
M. rutilum	czh-117^T	BBHF00000000.1	23.9
M. septicum	W4964^T	CBMO000000000.1	20.3
M. setense	ABO-M06^T	JTJW00000000.1	20.2
M. simiae	ATCC 25275^T	CBMJ000000000.2	23.4
M. smegmatis	ATCC 19420^T	LN831039.1	20.4
M. thermoresistibile	ATCC 19527^T	BCTB00000000.1	20.6
M. triplex	90-1019^T	CCAU000000000.1	24.9
M. tuberculosis	H37Rv^T	NC_000962.3	23.2
M. vaccae	ATCC 15483^T	BCRS00000000.1	20.7
M. vanbaalenii	PYR-1^T	NC_008726.1	20.4
M. vulneris	NLA000700772^T	CCBG000000000.1	20.4
M. yongonense	05-1390^T	NC_021715.1	25.8
Nocardia asteroides	NBRC 15531^T	BAFO00000000.2	19.7

Date: 21 DEC 2017 **Signature:**

BEI Resources Authentication

ATCC®, on behalf of BEI Resources, hereby represents and warrants that the material provided under this certificate has been subjected to the tests and procedures specified and that the results described, along with any other data provided in this certificate, are true and accurate to the best of ATCC®'s knowledge.

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