

***Mycobacterium simiae*, Strain CJ-49089**

**Catalog No. NR-50650**

**Product Description:** *Mycobacterium simiae* (*M. simiae*), strain CJ-49089 is of unknown origin.

**Lot<sup>1</sup>: 70001551**

**Manufacturing Date: 22JUL2016**

TEST	SPECIFICATIONS	RESULTS
<b>Phenotypic Analysis<sup>2</sup></b> Cellular morphology Colony morphology <sup>3</sup>  Growth rate Growth at 45°C Growth at 55°C Acid-fast stain Pigmentation in the dark (Scotochromogen) Photoinduction for 1 hour (Photochromogen) Nonchromogen (no pigment) Biochemical tests Catalase Catalase (semiquantitative) Catalase (68°C) Iron uptake Nitrate reduction Tween 80 hydrolysis Urease Growth in the presence of 5% sodium chloride Growth in the presence of thiophene-2-carboxylic acid hydrazide (TCH)	Gram-positive rods Report results  ≥ 7 days Negative Report results Positive (red colonies) Negative Positive Negative  Positive Report results Positive Negative Negative Negative Positive Negative Positive	Rods Circular, convex, entire, rough and cream (Figure 1) 7 days Negative Negative Positive (red colonies) <b>Positive<sup>4</sup></b> <b>Negative<sup>4</sup></b> Negative  Positive Positive Positive Negative Negative Negative Negative <b>Negative<sup>4</sup></b> <b>Positive<sup>4</sup></b> <b>Negative<sup>4</sup></b>
<b>Genotypic Analysis</b> Sequencing of 16S ribosomal RNA gene (~ 1480 base pairs)  Digital DNA-DNA hybridization (dDDH) <sup>5</sup>	≥ 99% sequence identity to <i>M. simiae</i> type strain (GenBank: GQ153280.1) ≥ 70% for species identification	100% sequence identity to <i>M. simiae</i> type strain (GenBank: GQ153280.1) <i>M. simiae</i> (99.9%) <sup>6,7</sup>
<b>Purity (post-freeze)</b> Middlebrook 7H10 agar with OADC enrichment <sup>8</sup>  Tryptic Soy agar <sup>8</sup>  Tryptic Soy agar with 5% defibrinated sheep blood <sup>8</sup>	Growth consistent with expected colony morphology Report results  Report results	Growth consistent with expected colony morphology Growth consistent with expected colony morphology Growth consistent with expected colony morphology
<b>Viability (post-freeze)<sup>3</sup></b>	Growth	Growth

<sup>1</sup>NR-50650 was produced by inoculation of the deposited material into Middlebrook 7H9 broth with ADC enrichment and grown for 7 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 7 days at 37°C in an aerobic atmosphere with 5% CO<sub>2</sub> to produce this lot.

<sup>2</sup>Phenotypic characterization of *M. simiae* 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 Magee, J. G. and A.C. Ward. "Family III. *Mycoacteriaceae* Chester 1897, 63<sup>AL</sup>." *Bergey's® Manual of Systematic Bacteriology, Volume Five.* (2012) Goodfellow, M., et al. (Ed.), Springer.

<sup>3</sup>7 days at 37°C in an aerobic atmosphere with 5% CO<sub>2</sub> on Middlebrook 7H10 agar with OADC enrichment

<sup>4</sup>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 a positive biochemical result is represented by >85% of strains tested being positive and a negative result is represented by <15% of strains tested being positive.

<sup>5</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." *Stand Genomic Sci.* 2 (2010): 117-134, PubMed: 21304684.

<sup>6</sup>The whole genome of *M. simiae*, strain CJ-49089 (Total Contig Length ~ 5.7 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>7</sup>*Mycobacterium simulans* is not sequenced in the dDDH database so it was not included in the analysis.

<sup>8</sup>Purity of this lot was assessed for 7 days at 37°C in an aerobic atmosphere with 5% CO<sub>2</sub>.

**Figure 1: Colony Morphology**



**Date:** 12 JUN 2017

**Signature:**



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