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SUPPORTING INFECTIOUS DISEASE RESEARCH

Certificate of Analysis for NR-50276

Brucella melitensis, Strain 16MΔ*vjbR*

Catalog No. NR-50276

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Product Description: *Brucella melitensis* (*B. melitensis*), strain 16MΔ*vjbR* is an attenuated vaccine strain that was adapted from *B. melitensis,* strain 16M.

Lot[1]: 70006632 **Manufacturing Date: 23JUN2017**

TEST	SPECIFICATIONS	RESULTS
Phenotypic Analysis		
Cellular morphology[2]	Report results	Gram-negative rods
Colony morphology[2]	Report results	Circular, low convex, entire, smooth and cream (Figure 1)
Motility[3]	Non-motile	Non-motile
Biochemical tests[4]		
Oxidase	Positive	Positive
Urease	Positive (> 5 minutes)	Positive (> 5 minutes)
Hydrogen sulfide production (lead acetate)	Positive	Positive
Arginine dihydrolase	Negative	Negative
Arabinose	Negative	Positive[5]
Glucose	Positive	Positive
Xylose	Positive[4]	Positive
Genotypic Analysis		
Sequencing of 16S ribosomal RNA gene (~ 1490 base pairs)	≥ 99% sequence identity to *B. melitensis*, strain 16M (GenBank: CP034103)	100% sequence identity to *B. melitensis*, strain 16M (GenBank: CP034103)
Digital DNA-DNA hybridization (dDDH)[6]	≥ 70% for species identification	*B. melitensis* (97.8%)[7,8]
Confirmation of deletion by Next-Generation Sequencing (illumina® MiSeq)	Δ*vjbR* (locus BMEII1116)	Δ*vjbR* (locus BMEII1116)[9]
Purity (post-freeze)[10]	Growth consistent with expected colony morphology	Growth consistent with expected colony morphology
Viability (post-freeze)[2]	Growth	Growth

[1]NR-50276 was produced by inoculation of the deposited material into Tryptic Soy broth and grown 2 days at 37°C in an aerobic atmosphere with 5% CO_2. Broth inoculum was added to Tryptic Soy agar with 5% defibrinated sheep blood agar kolles and grown 2 days at 37°C in an aerobic atmosphere with 5% CO_2 to produce this lot.

[2]3 days at 37°C in an aerobic atmosphere on Tryptic Soy agar

[3]Motility test was performed on Remel™ Motility Test Medium w/TTC indicator for 3 days at 37°C in an aerobic atmosphere with 5% CO_2.

[4]Specifications are based on the testing results for the parent strain (BEI Resources NR-256) and may not agree with the expected specifications for *B. melitensis*.

[5]Arabinose metabolism was tested three times and was positive each time for this strain. This result differs from parental strain, and may be a result of the genetic manipulation of this strain or a spontaneous mutation.

[6]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.

[7]The whole genome of *B. melitensis*, strain 16MΔ*vjbR* (Genome Total Length 3,270,279 base pairs) was sequenced using the Illumina® MiSeq® system and was assembled and analyzed with CLC Genomics Workbench Version 7.0.2.

[8]The dDDH testing was not able to distinguish between *Brucella* spp. (*abortus, canis, ceti, inopinata, melitensis, microti, neotomae, ovis, pinnipedialis, suis* and *vulpis*). All of these species produced scores >70% and thus, by the dDDH criteria, are considered the same species.

[9]Alignment of the NR-50276 DNA sequence to the strain 16M sequence illustrates the 760 base pair deletion described by the contributor (Arenas-Gamboa, A. M., et al. "Extended Safety and Efficacy Studies of the Attenuated *Brucella* Vaccine Candidates 16MΔ*vjbR* and S19Δ*vjbR* in the Immunocompromised IRF-1$^{-/-}$ Mouse Model." Clin. Vaccine Immunol. 19 (2012): 249-260. PubMed: 22169089.).

[10]Purity of this lot was assessed for 10 days at 37°C in an aerobic atmosphere with 5% CO_2 on Tryptic Soy agar.

BEI Resources
www.beiresources.org

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

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Figure 1: Colony Morphology

/Heather Couch/
Heather Couch 19 MAR 2019
Program Manager or designee, ATCC Federal Solutions

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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BEI Resources
www.beiresources.org

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