



Draft Genome Sequences of *Anaplasma marginale* Strains MEX-15-099-01 and MEX-31-096-01, Two Mexican Isolates with Different Degrees of Virulence

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ABSTRACT *Anaplasma marginale* is an intraerythrocytic bacterium that causes bovine anaplasmosis and is endemic in Mexico. In this work, we report two draft genome sequences of Mexican isolates from different geographical regions and with different degrees of virulence.

Anaplasma marginale is an intraerythrocytic rickettsial Gram-negative bacterium that causes clinical signs of bovine anaplasmosis, namely, fever, anemia, jaundice, weakness, and even respiratory distress. *A. marginale* is endemic in tropical and subtropical areas of the world, including Mexico (1). Only one genome sequence, that of the Mexican strain MEX-01-001-01 from Aguascalientes, has actually been reported (2). In 2000, García et al. evaluated the level of virulence of two Mexican strains, the high-virulence strain MEX-15-099-01 isolated from Texcoco, State of Mexico, and the low-virulence strain MEX-31-096-01 isolated from Tizimin, Yucatan (3). Also, in 2008, Rodríguez-Camarillo et al. reported that strain MEX-31-096-01 is used as a live vaccine (4). Here, we report the draft genome sequences of *A. marginale* strains MEX-15-099-01 and MEX-31-096-01, which were isolated from the blood of sick cattle and blood of asymptomatic cattle, respectively.

We used 200 μ l of bovine blood for each isolate to extract genomic DNA using the UltraClean DNA BloodSpin kit (Mo Bio Laboratories). Genomic DNA (2 μ g) for each isolate was sequenced using the MiSeq platform (Illumina), and the libraries were prepared by fragmenting the genomic DNA and ligating specialized adapters to both fragment ends (Arizona State University DNA Sequencing Core). We obtained two data sets of 605,370 (MEX-15-099-01) and 1,671,658 (MEX-31-096-01) 300-bp paired-end reads, which were reported to the SRA database.

The Illumina adapter sequences were removed from paired-end reads using the ILLUMINACLIP trimming step of Trimmomatic (version 0.36), with default settings (5). Low-quality bases were removed using the dynamictrim algorithm of the SolexaQA++ suite (version 3.1.7.1) (6) with a Phred quality score (Q) of <13. The resulting paired-end reads were *de novo* assembled using SPAdes (version 3.11.1) (7) with the following options: (i) only run the assembly module (-only-assembler), (ii) reduce the number of mismatches (-careful), and (iii) k-mer lengths between 21 and 127. Contigs of two Mexican strains were differentiated from contigs that belong to other organisms (i.e., bovine genomes) based on G+C content of each contig assessed using a Python script (https://github.com/FernandoMtzMx/GC_content_MultiFasta) (the *A. marginale* genomes reported in databases have a G+C content between 46 and 52%). Also, we aligned the sequences of each contig assembled with the NCBI Nucleotide (nr/nt) database using the BLASTN suite (8) and "*Anaplasma marginale*" as the organism name. Contigs with an alignment coverage of greater than 50% and an identity of greater than

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TABLE 1 Statistics of the two assembled genomes

Statistic	Value for strain:	
	MEX-15-099-01	MEX-31-096-01
Genome size (bp)	1,169,440	1,176,579
No. of contigs	32	43
N_{50} length (bp)	85,955	62,699
Coverage (×)	~30	~19
G+C content (%)	49.79	49.79
GenBank accession no.	VTWW00000000	VTWW00000000
No. of genes	1,185	1,204
No. of CDS ^a	1,145	1,164

^a CDS, coding sequences.

70% belong to *A. marginale* genomes. The features of the two draft genomes were evaluated using QUAST (version 4.6.2) (9). The assembly statistics are shown in Table 1.

The draft genomes of strains MEX-15-099-01 and MEX-31-096-01 were annotated automatically using the Rapid Annotations using Subsystems Technology (RAST; version 2.0) server (10). The annotation statistics are shown in Table 1. The 16S rRNA gene sequences of two Mexican strains were obtained using the RNAmmer server (version 1.2) (11), and each has a length of 1,491 bp, as well as 100% alignment coverage and 100% identity with the 16S rRNA gene sequence of *A. marginale* strain St. Maries (GenBank accession number [CP000030](#)).

Data availability. This whole-genome shotgun project has been deposited at DDBJ/ENA/GenBank under the accession numbers [VTWW00000000](#) and [VTWW00000000](#) for MEX-15-099-01 and MEX-31-096-01, respectively. The versions described in this paper are versions VTWW01000000 and VTWW01000000 for MEX-15-099-01 and MEX-31-096-01, respectively. The publicly available raw data numbers (SRA) are [SRR10197851](#) and [SRR10198112](#) for MEX-15-099-01 and MEX-31-096-01, respectively.

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REFERENCES

- Rodríguez SD, García Ortiz MA, Jiménez Ocampo R, Vega y Murguía CA. 2009. Molecular epidemiology of bovine anaplasmosis with a particular focus in Mexico. *Infect Genet Evol* 9:1092–1101. <https://doi.org/10.1016/j.meegid.2009.09.007>.
- Quiroz Castañeda RE, Amaro-Estrada I, Martínez-Ocampo F, Rodríguez-Camarillo S, Dantán González E, Cobaxin-Cárdenas M, Preciado-de la Torre JF. 2018. Draft genome sequence of *Anaplasma marginale* strain Mex-01-001-01, a Mexican strain that causes bovine anaplasmosis. *Microbiol Resour Announc* 7:e01101-18. <https://doi.org/10.1128/MRA.01101-18>.
- García Ortiz MA, Aboytes Torres R, Hernández Salgado G, Cantó Alarcón JG, Rodríguez SD. 2000. *Anaplasma marginale*: diferentes grados de virulencia en dos aislados mexicanos. *Vet Méx* 31:157–160.
- Rodríguez Camarillo SD, García Ortiz MA, Rojas Ramírez EE, Cantó Alarcón GJ, Preciado de la Torre JF, Rosario Cruz R, Ramos Aragón JA, Aboytes Torres R. 2008. *Anaplasma marginale* Yucatan (Mexico) strain: assessment of low virulence and potential use as a live vaccine. *Ann N Y Acad Sci* 1149:98–102. <https://doi.org/10.1196/annals.1428.067>.
- Bolger AM, Lohse M, Usadel B. 2014. Trimmomatic: a flexible trimmer for Illumina sequence data. *Bioinformatics* 30:2114–2120. <https://doi.org/10.1093/bioinformatics/btu170>.
- Cox MP, Peterson DA, Biggs PJ. 2010. SolexaQA: at-a-glance quality assessment of Illumina second-generation sequencing data. *BMC Bioinformatics* 11:485. <https://doi.org/10.1186/1471-2105-11-485>.
- Bankevich A, Nurk S, Antipov D, Gurevich AA, Dvorkin M, Kulikov AS, Lesin VM, Nikolenko SI, Pham S, Prjibelski AD, Pyshkin AV, Sirotkin AV, Vyahhi N, Tesler G, Alekseyev MA, Pevzner PA. 2012. SPAdes: a new genome assembly algorithm and its applications to single-cell sequencing. *J Comput Biol* 19:455–477. <https://doi.org/10.1089/cmb.2012.0021>.
- Altschul SF, Gish W, Miller W, Myers EW, Lipman DJ. 1990. Basic local alignment search tool. *J Mol Biol* 215:403–410. [https://doi.org/10.1016/S0022-2836\(05\)80360-2](https://doi.org/10.1016/S0022-2836(05)80360-2).
- Gurevich A, Saveliev V, Vyahhi N, Tesler G. 2013. QUAST: quality assessment tool for genome assemblies. *Bioinformatics* 29:1072–1075. <https://doi.org/10.1093/bioinformatics/btt086>.
- Aziz RK, Bartels D, Best AA, DeJongh M, Disz T, Edwards RA, Formsma K, Gerdes S, Glass EM, Kubal M, Meyer F, Olsen GJ, Olson R, Osterman AL, Overbeek RA, McNeil LK, Paarmann D, Paczian T, Parrello B, Pusch GD, Reich C, Stevens R, Vassieva O, Vonstein V, Wilke A, Zagnitko O. 2008. The RAST server: Rapid Annotations using Subsystems Technology. *BMC Genomics* 9:75. <https://doi.org/10.1186/1471-2164-9-75>.
- Lagesen K, Hallin P, Rødland EA, Staerfeldt H-H, Rognes T, Ussery DW. 2007. RNAmmer: consistent and rapid annotation of ribosomal RNA genes. *Nucleic Acids Res* 35:3100–3108. <https://doi.org/10.1093/nar/gkm160>.