Draft Genome Sequences of *Mycoplasma mycoides* subsp. *mycoides* Strains APF9 and AP108, Isolated in Nigeria in 2014 to 2016

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**ABSTRACT** *Mycoplasma mycoides* subsp. *mycoides* is the etiological agent of contagious bovine pleuropneumonia (CBPP). While several findings on CBPP prevalence in Nigeria were documented, no data were reported about the genomic characterization of Nigerian *M. mycoides* subsp. *mycoides* strains. Here, we present the draft genome sequences of two novel *M. mycoides* subsp. *mycoides* strains isolated in Nigeria.

*Mycoplasma mycoides* subsp. *mycoides* is the causative agent of contagious bovine pleuropneumonia (CBPP), a severe contagious respiratory disease affecting cattle, which is included among the Office International des Epizooties (OIE) diseases (1). Most countries have eradicated CBPP (http://www.oie.int/en/animal-health-in-the-world/official-disease-status/cbpp), but the disease still remains endemic in sub-Saharan Africa, including Nigeria, where it causes, directly or indirectly, economic losses of about $2 billion annually (2).

Epidemiological studies were performed in the northeastern region of Nigeria (3, 4), but no extensive work was carried out on the genomic characterization of Nigerian *M. mycoides* subsp. *mycoides* isolates, which would be of great importance in order to clarify the relationship among strains circulating in different regions of the country, providing support to CBPP outbreak investigations and disease control.

The draft genome sequences of two novel *M. mycoides* subsp. *mycoides* strains, named APF9 and AP108, are presented here. The first strain, APF9, was isolated in 2014 from pleural fluid of a cow showing pathological lesions referring to CBPP at slaughter in the Yola Modern Abattoir (Adamawa State, Nigeria) (5). The AP108 strain was isolated in 2016 from pleural fluid of a regularly slaughtered cow in the Yola Modern Abattoir.

The isolates were grown at 37°C under 5% CO2 in pleuropneumonia-like organism (PPLO) broth and agar (Difco) supplemented as previously described (6). The genomic DNA was extracted using a Maxwell 16-cell DNA purification kit (Promega), and a PCR-based test (7) was used for the identification of *M. mycoides* subsp. *mycoides*. Library preparation was carried out using a Nextera XT library prep kit (Illumina, Inc.), according to the manufacturer’s manual. The libraries were loaded onto an Illumina 300-cycle NextSeq 500/550 mid output reagent cartridge 2 kit and sequenced on an Illumina NextSeq 500 platform, producing 150-bp paired-end reads.

A total of 1,782,894 and 1,146,316 reads were obtained for the APF9 and AP108 strains, respectively, corresponding to theoretical coverages of 450× and 285×, respectively (average read length, 150 bp). After trimming performed using Trimmomatic 0.35 (8) (ILLUMINACLIP:NexteraPE-PE:2:30:10, LEADING:25, TRAILING:25, SLIDINGWINDOW:
de novo assembly was carried out using SPAdes 3.11 (9), with default parameters for Illumina reads. Contigs longer than 200 bp were ordered by ABACAS 1.3 (10) using the genome of M. mycoides subsp. mycoides strain T1/44 (GenBank accession number CP014346) as a reference. Gaps were then filled using Pilon 1.23 (11) and GapFiller 2.1.1 (12).

The final assemblies, consisting of 3 contigs for the APF9 strain (total length, 1,203,001 bp; GC content, 23.96%; contig lengths, 999,440, 124,562, and 78,999 nucleotides) and 2 contigs for the AP108 strain (total length, 1,203,800 bp; GC content, 23.95%; contig lengths, 1,124,870 and 78,930 nucleotides), were submitted to GenBank, and annotation using the Prokaryotic Genome Annotation Pipeline (PGAP) pipeline (13) was requested. Genome annotation returned 1,159 genes for both the APF9 (including 936 protein-coding genes and 185 pseudogenes) and AP108 (including 935 protein-coding genes and 185 pseudogenes) strains. Two rRNA operons were annotated for each strain, and 30 tRNAs and 3 noncoding RNAs (ncRNAs) were identified. No plasmids were found in the APF9 and AP108 strains.

Data availability. The genome sequences were deposited in GenBank under the following accession numbers: VCPG00000000 (strain APF9) and VCPH00000000 (strain AP108). The Illumina raw reads were deposited in the NCBI Sequence Read Archive (SRA) under the accession numbers SAMN11775870 (strain APF9) and SAMN11775871 (strain AP108).

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REFERENCES

1. World Organisation for Animal Health. 2014. Contagious bovine pleuropneumonia, p 1–16. In Manual of diagnostic tests and vaccines for terrestrial animals, 6th ed. Office International des Epizooties, Paris, France.

2. Food and Agricultural Organization (FAO). 2004. Proceedings of the 3rd Meeting of the FAO-OIE-OAU/IAEA Consultative Group on CBPP Towards Sustainable CBPP Control Programmes for Africa, 12 to 14 November 2003, Rome, Italy, p 1–46.

3. Aliyu MM, Obi TU, Egwu GO. 2000. Prevalence of contagious bovine pleuropneumonia in northern Nigeria. Prev Vet Med 47:263–269. https://doi.org/10.1016/S0167-5877(00)00170-7.

4. Mailafiya S, Onakpa MM, Dandan KP. 2010. A ten-year retrospective study on the prevalence of ruminant diseases encountered at the ministry of the Federal Capital Territory Veterinary Clinic Gwagwalada, Abuja, Nigeria. Sahel J Vet Sci 9:4–6.

5. Musa JA, Bake JOO, Kazeem HM, Nwankpa ND, Di Provvido A, Sacchini F, Zilli K, Abass A, Scacchia M, Pini A. 2016. Molecular detection of Nigerian field isolates of Mycoplasma mycoides subsp. mycoides as causative agents of contagious bovine pleuropneumonia. Int J Vet Sci Med 4:46–53. https://doi.org/10.1016/j.jivsm.2016.10.007.

6. Cetinkaya B, Ongor H, Karahan M, Kalender H, Lorenzon S, Thiaucourt F. 2003. Abattoir-based survey of contagious bovine pleuropneumonia in cattle in Turkey. Vet Rec 152:254–258. https://doi.org/10.1136/vir.152.9.254.

7. Bashiruddin JR, Taylor TK, Gould AR. 1994. A PCR-based test for the specific identification of Mycoplasma mycoides subspecies mycoides SC. J Vet Diagn Invest 6:428–434. https://doi.org/10.1177/104063879400600405.

8. 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.

9. Bankevich A, Nurk S, Antipov D, Gurevich AA, Dvorkin M, Kulikov AS, Lesin VM, Nikolenko SI, Pham S, Prijibelski 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.

10. Assefa S, Keane TM, Otto TD, Newbold C, Berriman M. 2009. ABACAS: algorithm-based automatic contiguation of assembled sequences. Bioinformatics 25:1968–1969. https://doi.org/10.1093/bioinformatics/btp347.

11. Walker BJ, Abeel T, Shea T, Priest M, Abouelliel A, Sakthikumar S, Cuomo CA, Zeng Q, Wortman J, Young SK, Earl AM. 2014. Pilon: an integrated tool for comprehensive microbial variant detection and genome assembly improvement. PLoS One 9:e112963. https://doi.org/10.1371/journal.pone.0112963.

12. Boetzer M, Pirovano W. 2012. Toward almost closed genomes with GapFiller. Genome Biol 13:R56. https://doi.org/10.1186/gb-2012-13-6-r56.

13. Tatusova T, Di Cuccio M, Badreddin A, Chevetmin V, Nawrocki EP, Zaslavsky L, Lomsadze A, Nikolenko SI, Pham S, Prijibelski AD, Pyshkin AV, Sirotkin AV, Vyahhi N, Tesler G, Alekseyev MA, Pevzner PA. 2016. NCBI Prokaryotic Genome Annotation Pipeline. Nucleic Acids Res 44:6614–6624. https://doi.org/10.1093/nar/gkw569.