# Draft Genome Sequence of *Acholeplasma laidlawii* isolated from the conjunctiva of a heifer with Infectious Bovine Keratoconjunctivitis

**Gioia Gloria[1], Addis Maria Filippa[2], Goodman Laura[1], Mitchell Patrick[1], Thompson Belinda[1], Goodrich Erin[1], Moroni Paolo[1,2]**

[1] Animal Health Diagnostic Center, Cornell University, Ithaca NY

[2] Università degli Studi di Milano, Dipartimento di Medicina Veterinaria, 26900 Lodi, Italy

## ABSTRACT

*Acholeplasma laidlawii* can be isolated from cattle environments and different body sites of bovines. It is still under evaluation if *A. laidlawii* may act as a primary pathogen. Here, we present the whole-genome sequence of *A. laidlawii* isolated from the conjunctiva of a heifer with Infectious Bovine Keratoconjunctivitis.

## ANNOUNCEMENT

Acholeplasmas are cell wall-less bacteria belonging to the class Mollicutes, order Acholeplasmatales, and classified in the family of Acholeplasmataceae. *Acholeplasma spp.* are described as saprophytes found in soil, compost, and wastewater or commensals distributed in vertebrates, insects, and plants (6).

Within the *Acholeplasma* genus, *Acholeplasma laidlawii* is the best-studied species. *A. laidlawii* is often found in dairy and beef cattle environments. It has been isolated from different body sites of bovines, including mastitic milk, bulk tank milk, aborted fetus, semen, preputial samples, nasal secretions, pneumonia, and healthy lungs (7, 4). One trial performed by Pugh et al. in 1976 (8) demonstrated the ability of *A. laidlawii* to establish clinical signs of infectious bovine keratoconjunctivitis in calves after experimental induction. Other publications exclude the role of *A. laidlawii* as the causative agent of keratoconjunctivitis. Still, the authors state that the simultaneous presence of *A. laidlawii* with other major pathogens may contribute to lesion severity (1).

Here, we announce the draft genome sequence of *A. laidlawii* strain QMP CG1-1743 isolated from the conjunctival swab of a 6-9 months' heifer affected with Infectious Bovine Keratoconjunctivitis and showing watery eyes and corneal opacities. The heifer under testing

was from an organic herd; it was not treated with any antibiotics but was administered plasma eye drops as treatment. The initial isolation of the strain PG-8A was carried out by standard procedures for mycoplasma culturing, which include streaking on modified Hayflick agar medium and incubation for up to 7 days at 37°C with CO2 enrichment. Well defined positive colonies showing typical mycoplasma morphology were submitted to an initial speciation test that includes a PCR amplification of the 16S-23S internal transcribed spacer (ITS) followed by Sanger sequencing of the amplicons (2).

The isolate was then sub-cultured in modified Hayflick medium, incubated for 3 days at 37°C with CO2, and pelleted by centrifugation at 13,000 g for 10 minutes. DNA was directly extracted from the pellet using MagMAX core nucleic acid purification kit (Applied Biosystems, Foster City, CA). DNA concentration was measured with a Qubit 3.0 fluorometer (Life Technologies, MD). The isolate was sequenced on an Illumina MiSeq using 2 x 250 bp reads, and read quality was assessed using FastQC (https://www.bioinformatics.babraham.ac.uk/projects/fastqc/) (5). A total of 406,301 paired reads were generated with an average Phred score of 37.35 for forward reads and 37.12 for reverse reads. Raw reads were assembled using SKESA v. 2.3.0 (9), assembly quality was checked using QUAST v. 5.0.2 (3), and reads were mapped back to the assembly to identify coverage depth with BBMap v. 38.58 (http://sourceforge.net/projects/bbmap/). Total assembly length was 1,329,497 bp with 55 contigs, an N50 of 75,018, and 31.74% GC content. The average read depth was 108×. Average nucleotide identity was computed against all Acholeplasma genomes available from GenBank (accessed July 7, 2020) using fastANI v. 1.3. The highest ANI was 93.8% with *A. laidlawii* strain MDBK/IPV (GCA_001730135.1). ANIs in comparison to three assemblies of the A. laidlawii type strain (GCA_000018785.1, GCA_900476025.1, and GCA_003385765.1) were 93.2–93.3%, indicating that this isolate is divergent from previously sequenced *A. laidlawii* isolates and might represent a distinct lineage.

**Data availability.** Sequencing data has been submitted to NCBI under BioSample SAMN16288725.

## ACKNOWLEDGMENTS

The research was supported by the Hatch Grant “Mycoplasma in dairy farms: evaluation of a novel enrichment real-time PCR for rapid management” 2018-2020 Moroni (PI). NYC-2018-19-131i). We also thank Renee Anderson and Rebecca Franklin-Guild for their excellent technical support. Sequencing capacity used for this study was funded (FOA PAR-18-604) by the U.S. Food and Drug Administration’s Veterinary Laboratory Investigation and Response Network (FDA Vet-LIRN) under grant 1U18FD006716.

## REFERENCES

1. Wilcock BP, Bradley LN. 2016. Jubb, Kennedy & Palmer's Pathology of Domestic Animals: Volume 1 (Sixth Edition) Chapter 5: 41.

2. Gioia G, Werner B, Nydam DV, Moroni P. 2016. Validation of a mycoplasma molecular diagnostic test and distribution of mycoplasma species in bovine milk among New York State dairy farms. J Dairy Sci 99(6):4668-4677. doi: 10.3168/jds.2015-10724. Epub 2016 Mar 24. PubMed PMID: 27016831.

3. Gurevich A, Saveliev V, Vyahhi N, Tesler G. 2013. QUAST: quality assessment tool for genome assemblies. Bioinformatics 29:1072–1075. doi:10.1093/bioinformatics/btt086.

4. Kahane I, Adoni A. 2012. Rapid Diagnosis of Mycoplasmas. Springer Science & Business Media. Dec 6:78.

5. Jain C, Rodriguez-R LM, Phillippy AM, Konstantinidis KT, Aluru S. 2018. High throughput ANI analysis of 90K prokaryotic genomes reveals clear species boundaries. Nat Commun 9:5114. doi:10.1038/s41467-018-07641-9.

6. Kube M, Siewert C, Migdoll AM, Duduk B, Holz S, Rabus R, Seemüller E, Mitrovic J, Müller I, Büttner C, Reinhardt R. 2014. Analysis of the complete genomes of *Acholeplasma brassicae*, *A. palmae* and *A. laidlawii* and their comparison to the obligate

parasites from 'Candidatus Phytoplasma'. J Mol Microbiol Biotechnol. 24(1):19-36. doi: 10.1159/000354322. Epub 2013 Oct 18. PubMed PMID: 24158107.

7. DOIG PA. 1981. Bovine Genital Mycoplasmosis. Can. vet. J. 22: 339-343

8. Pugh GW, Hughes DE, Schulz VD. 1976. Infectious bovine keratoconjunctivitis: experimental induction of infection in calves with mycoplasmas and *Moraxella bovis*. Am J Vet Res. 37(5):493-5. PubMed PMID: 1275332.

9. Souvorov A, Agarwala R, Lipman DJ. 2018. SKESA: strategic k-mer extension for scrupulous assemblies. Genome Biol 19:153. doi:10.1186/s13059-018-1540-z.