

# Draft Genome Sequences of *Mycoplasma alkalescens*, *Mycoplasma arginini*, and *Mycoplasma bovis*, Three Species with Equivocal Pathogenic Status for Cattle

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**We report here the draft genome sequences of *Mycoplasma alkalescens*, *Mycoplasma arginini*, and *Mycoplasma bovis*. These three species are regularly isolated from bovine clinical specimens, although their role in disease is unclear.**

Received 26 April 2013 Accepted 1 May 2013 Published 13 June 2013

**Citation** Manso-Silván L, Tardy F, Baranowski E, Barré A, Blanchard A, Breton M, Couture C, Citti C, Dordet-Frisoni E, Dupuy V, Gaurivaud P, Jacob D, Lemaitre C, Nikolski M, Nouvel L-X, Poumarat F, Thébaud P, Theil S, Thiaucourt F, Sirand-Pugnet P. 2013. Draft genome sequences of *Mycoplasma alkalescens*, *Mycoplasma arginini*, and *Mycoplasma bovis*, three species with equivocal pathogenic status for cattle. *Genome Announc.* 1(3):e00348-13. doi:10.1128/genomeA.00348-13.

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*Mycoplasma alkalescens*, *Mycoplasma arginini*, and *Mycoplasma bovis* are bacteria of the class *Mollicutes* clustered within the hominis phylogenetic group. They have been associated with disease in cattle, but their contribution to pathogenesis remains unclear. *M. alkalescens*, described in 1973 (1), has been reported from mastitis in cattle and from arthritis, otitis, and pneumonia in calves (2–5). In pneumonic calves, *M. alkalescens* is often associated with *Mycoplasma bovis*, but recent recurrent isolation in the United Kingdom in the absence of other pathogens suggests it may constitute an emerging mycoplasma (6). *M. arginini*, characterized in 1968 (7), is a much more ubiquitous species isolated from a broad collection of mammalian hosts (8). It has been associated with various pathologies in ruminants and is often found in association with *M. bovis* in cattle (3). However, its pathogenicity has never been established (9), and this species is

best known as a frequent contaminant of eukaryotic cell cultures (10). *M. bovis*, characterized in 1955 (11), comprises the strains of the *Mycoplasma* ovine/caprine serogroup 11, reassigned in 2008 (12). It has been associated with reproductive disorders in ruminants (2, 13, 14) and has proven to induce pneumonia in gnotobiotic calves (9).

Genome sequences of the most relevant mycoplasmal bovine pathogens (*Mycoplasma mycoides* subsp. *mycoides* and *M. bovis*) have already been published. To widen the availability of genome data from mycoplasmas of bovine origin, we present here the genome sequences of *M. alkalescens*, *M. arginini*, and *M. bovis*.

Selected strains were isolated in France from lung tissue samples from calves with pneumonia. *M. alkalescens* strain 14918 and *M. arginini* strain 7264 were isolated in 2007, the latter being

TABLE 1 General properties of the three genome sequences

Characteristic	<i>M. alkalescens</i> 14918	<i>M. arginini</i> 7264	<i>M. bovis</i> 51080
No. of contigs >500 bp	20	18	42
Median coverage	126×	158×	44×
GenBank accession no.	AMWK000000000	AORG000000000	AORH000000000
Genome size (bp)	771,939	615,621	862,247
G+C (%)	25.56	26.22	28.96
Gene density (%)	85.18	90.24	88.5
No. of CDSs <sup>a</sup>	601	513	677
No. of pseudogenes	33	9	24
No. of structural RNA genes	37	36	36

<sup>a</sup> CDSs, coding sequences.

found in association with *Mannheimia haemolytica*. *M. bovis genitalium* strain 51080 was isolated in 2009 from a septicemic calf, again concomitantly with *M. haemolytica*. Whole-genome sequences were obtained using a combination of Illumina (single reads) and 454 (mate paired with 8-kb insert size). Assembly was performed using Newbler 2.3, and annotation was conducted using a customized version of the CAAT-Box platform (15), with automatic preannotation for coding sequences followed by expert validation, as detailed previously (16). Genome analysis and comparisons were mainly conducted using the MolliGen 3.0 platform (17).

The general properties of the three genomes are shown in Table 1. Sequences related to integrative conjugative elements were found in both *M. alkalescens* and *M. bovis genitalium*, whereas a prophage, similar to that previously described in the small ruminant pathogen *Mycoplasma agalactiae* (18), was identified in *M. bovis genitalium*. These mobile genetic elements constitute an important driving force of genome plasticity and may be associated with horizontal gene transfer among *Mycoplasma* species sharing the same habitat (16, 19).

Comparative genome analysis of mycoplasmas of bovine origin displaying diverse pathogenicity, as well as host and tissue tropism, will improve our understanding of the evolution of bovine mycoplasmas and will pave the way for unraveling the genetic basis of mycoplasma pathogenicity and host specificity.

**Nucleotide sequence accession numbers.** Draft genome sequences of *M. alkalescens*, *M. arginini*, and *M. bovis genitalium* were deposited as Whole-Genome Shotgun projects at GenBank under the accession no. [AMWK000000000](https://www.ncbi.nlm.nih.gov/nuccore/AMWK000000000), [AORG000000000](https://www.ncbi.nlm.nih.gov/nuccore/AORG000000000), and [AORH000000000](https://www.ncbi.nlm.nih.gov/nuccore/AORH000000000), respectively.

## ACKNOWLEDGMENTS

Financial support was provided by the EVOLMYCO project (ANR-07-GMGE-001) from ANR to Alain Blanchard (Principal Investigator [PI]), Franois Thiaucourt (Co-PI), Franois Poumarat (Co-PI), and Christine Citti (Co-PI).

## REFERENCES

1. Leach RH. 1973. Further studies on classification of bovine strains of *Mycoplasmatales*, with proposals for new species, *Acholeplasma modicum* and *Mycoplasma alkalescens*. *J. Gen. Microbiol.* 75:135–153.
2. Ayling RD, Bashiruddin SE, Nicholas RA. 2004. *Mycoplasma* species and related organisms isolated from ruminants in Britain between 1990 and 2000. *Vet. Rec.* 155:413–416.
3. Chazel M, Tardy F, Le Grand D, Calavas D, Poumarat F. 2010. Mycoplasmoses of ruminants in France: recent data from the national surveillance network. *BMC Vet. Res.* 6:32.
4. Kokotovic B, Friis NF, Ahrens P. 2007. *Mycoplasma alkalescens* demonstrated in bronchoalveolar lavage of cattle in Denmark. *Acta Vet. Scand.* 49:2.
5. Lamm CG, Munson L, Thurmond MC, Barr BC, George LW. 2004. *Mycoplasma* otitis in California calves. *J. Vet. Diagn. Invest.* 16:397–402.
6. Nicholas RA, Ayling RD, McAuliffe L. 2008. Bovine respiratory disease, p 132–168. *In* *Mycoplasma* diseases of ruminants. CAB International, Wallingford, United Kingdom.
7. Barile MF, DelGiudice RA, Carski TR, Gibbs CJ, Morris JA. 1968. Isolation and characterization of *Mycoplasma arginini*: spec. nov. *Proc. Soc. Exp. Biol. Med.* 129:489–494.
8. Tully JG, Whitcomb RF. 1979. The Mycoplasmas, vol. II. Human and animal Mycoplasmas. Academic Press, New York, NY.
9. Gourlay RN, Howard CJ, Thomas LH, Wyld SG. 1979. Pathogenicity of some *Mycoplasma* and *Acholeplasma* species in the lungs of gnotobiotic calves. *Res. Vet. Sci.* 27:233–237.
10. Drexler HG, Uphoff CC. 2002. *Mycoplasma* contamination of cell cultures: incidence, sources, effects, detection, elimination, prevention. *Cytotechnology* 39:75–90.
11. Freundt EA. 1955. The classification of the pleuropneumoniae group of organisms (*Borrelomycetales*). *Int. Bull. Bacteriol. Nomencl. Taxon.* 5:67–78.
12. Nicholas RA, Lin YC, Sachse K, Hotzel H, Parham K, McAuliffe L, Miles RJ, Kelly DP, Wood AP. 2008. Proposal that the strains of the *Mycoplasma* ovine/caprine serogroup 11 be reclassified as *Mycoplasma bovis genitalium*. *Int. J. Syst. Evol. Microbiol.* 58:308–312.
13. Ghanem ME, Higuchi H, Tezuka E, Ito H, Devkota B, Izaike Y, Osawa T. 2013. *Mycoplasma* infection in the uterus of early postpartum dairy cows and its relation to dystocia and endometritis. *Theriogenology* 79:180–185.
14. Nicholas RA, Ayling RD, McAuliffe L. 2008. Reproductive diseases of cattle, p 208–224. *In* *Mycoplasma* diseases of ruminants. CAB International, Wallingford, United Kingdom.
15. Frangul L, Glaser P, Rusniok C, Buchrieser C, Duchaud E, Dehoux P, Kunst F. 2004. CAAT-Box, contigs-assembly and annotation Tool-box for genome sequencing projects. *Bioinformatics* 20:790–797.
16. Thiaucourt F, Manso-Silvan L, Salah W, Barbe V, Vacherie B, Jacob D, Breton M, Dupuy V, Lomenech AM, Blanchard A, Sirand-Pugnet P. 2011. *Mycoplasma mycoides*, from “mycoides Small Colony” to “capri”. A microevolutionary perspective. *BMC Genomics* 12:114.
17. Barre A, de Daruvar A, Blanchard A. 2004. MolliGen, a database dedicated to the comparative genomics of Mollicutes. *Nucleic Acids Res.* 32:D307–D310.
18. Tardy F, Baranowski E, Nouvel LX, Mick V, Manso-Silvan L, Thiaucourt F, Thebault P, Breton M, Sirand-Pugnet P, Blanchard A, Garnier A, Gibert P, Game Y, Poumarat F, Citti C. 2012. Emergence of atypical *Mycoplasma agalactiae* strains harboring a new prophage and associated with an alpine wild ungulate mortality episode. *Appl. Environ. Microbiol.* 78:4659–4668.
19. Sirand-Pugnet P, Lartigue C, Marena M, Jacob D, Barre A, Barbe V, Schenowitz C, Mangenot S, Couloux A, Segurens B, de Daruvar A, Blanchard A, Citti C. 2007. Being pathogenic, plastic, and sexual while living with a nearly minimal bacterial genome. *PLoS Genet.* 3:e75.