

Genome Sequence of *Mycoplasma putrefaciens* Type Strain KS1

Michael J. Calcutt* and Mark F. Foecking

Department of Veterinary Pathobiology, University of Missouri, Columbia, Missouri 65211

Received 24 August 2011/Accepted 29 August 2011

***Mycoplasma putrefaciens* is a causative agent of contagious agalactia in goats. Reported herein is the complete genome sequence of the *M. putrefaciens* type strain KS1.**

Contagious agalactia (CA) is a small-ruminant mycoplasmosis of significant consequence that is reportable to the World Organization for Animal Health (1). The syndrome includes mastitis and agalactia which may be accompanied by conjunctivitis and polyarthritis. Four etiological agents have been identified (*Mycoplasma agalactiae*, *Mycoplasma capricolum* subsp. *capricolum*, *Mycoplasma mycoides* subsp. *capri*, and *Mycoplasma putrefaciens*), but the virulence attributes of these species are incompletely known. With the genome sequence of three species available, that of the *M. putrefaciens* type strain was determined to provide clues to the molecular basis of pathogenesis as well as to gain insight into the evolution of the *M. mycoides* cluster, a clade of taxa to which *M. putrefaciens* is the most closely related pathogen (3).

The genome sequence of *M. putrefaciens* KS1 was determined by 454 Titanium GS-FLX-based pyrosequencing (performed at The Genome Center, Washington University, St. Louis, MO). The resulting sequence reads were assembled into 8 contigs (454 Newbler software) with 144× genome coverage. Gap closure was completed by PCR amplification and Sanger sequencing. Open reading frames (ORFs; Glimmer), tRNAs (tRNAscan-SE), and rRNAs (blastn) were identified and provisionally annotated using the bioinformatics pipeline of the Institute of Genome Sciences (University of Maryland, Baltimore, MD), prior to manual curation of each gene. The single circular chromosome is comprised of 832,603 bp with a G+C content of 26.9%. The genome contains 725 genes, of which 686 contain predicted ORFs (including 36 pseudogenes with confirmed degeneracy, truncation, or frameshift mutations), and 39 correspond to structural RNAs. No plasmids, prophages, or integrative conjugative elements (ICE) were identified, although 6 pseudogenes for transposases and ICE-contained ORFs were detected. Most ORFs had the highest sequence identity to those of the *M. mycoides* cluster, consistent with the phylogenetic grouping of these species.

Despite the *M. putrefaciens* chromosome being >170 kb smaller than other sequenced genomes of the *M. mycoides* cluster, examples of apparent gene expansion were noted

among the gene set encoding the surface lipoprotein repertoire. A paralogous family of 3 tandemly arrayed *lppB* genes (2) and 3 clustered pairs of conserved lipoprotein genes are arrangements that have not been identified in other *Mycoplasma* genomes. Of the 40 lipoprotein genes detected, 11 are predicted to encode *M. putrefaciens*-specific surface antigens based on the absence of significant matches in the current databases.

M. putrefaciens is unique among *Mycoplasma* species in its ability to produce a putrid odor during culture (5). Query of the deduced proteome for enzymes that might endow this trait resulted in the identification of a gene encoding L-methionine gamma-lyase (MGL). One reaction product of this pyridoxal phosphate-dependent enzyme is methanethiol (4). Based on the odor of this gas and the absence of MGL-encoding genes in any of the sequenced mycoplasmal genomes, MGL is postulated to contribute to the malodor that is the species epithet.

The annotated genome sequence provides a reference for comparative analysis of CA-causing pathogens and is a platform for postgenomic studies of *M. putrefaciens* pathobiology.

Nucleotide sequence accession number. The genome sequence is available from GenBank under accession number CP003021.

We thank Mary Brown, University of Florida, for providing *M. putrefaciens* KS1.

This work was funded in part by the USDA-ARS Program for Prevention of Animal Infectious Diseases (grant 1940-32000-039-08S) and by the University of Missouri Faculty Council.

REFERENCES

1. **Bergonier, D., X. Berthelot, and F. Poumarat.** 1997. Contagious agalactia of small ruminants: current knowledge concerning epidemiology, diagnosis and control. *Rev. Sci. Tech.* **16**:848–873.
2. **Djordjevic, S. P., E. M. Vilei, and J. Frey.** 2003. Characterization of a chromosomal region of *Mycoplasma* sp. bovine group 7 strain PG50 encoding a glycerol transport locus (*gtsABC*). *Microbiology* **149**:195–204.
3. **Manso-Silván, L., X. Perrier, and F. Thiaucourt.** 2007. Phylogeny of the *Mycoplasma mycoides* cluster based on analysis of five conserved protein-coding sequences and possible implications for the taxonomy of the group. *Int. J. Syst. Evol. Microbiol.* **57**:2247–2258.
4. **Nakayama, T., et al.** 1984. Purification of bacterial L-methionine γ -lyase. *Anal. Biochem.* **138**:421–424.
5. **Tully, J. G., M. F. Barile, D. G. Edward, T. S. Theodore, and H. Erno.** 1974. Characterization of some caprine mycoplasmas, with proposals for new species, *Mycoplasma capricolum* and *Mycoplasma putrefaciens*. *J. Gen. Microbiol.* **85**:102–120.

* Corresponding author. Mailing address: Department of Veterinary Pathobiology, 201 Connaway Hall, University of Missouri, Columbia, MO 65211. Phone: (573) 882-1291. Fax: (573) 884-5414. E-mail: calcuttm@missouri.edu.