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Genome Analysis of a *Mycoplasma hyorhinis* Strain Derived from a Primary Human Melanoma Cell Line▽

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The complete genome of *Mycoplasma hyorhinis* strain MCLD has been sequenced and annotated. This genome differs by the inversion of a 14.4-kb and a 3.7-kb fragment and the deletion of a 9.9-kb fragment from *M. hyorhinis* strain HUB-1, isolated from swine respiratory tract. The genome revealed 778 coding sequences (CDSs), with a limited number of *vlp* genes encoding variable surface lipoproteins.

Mycoplasma hyorhinis is a swine pathogen causing respiratory diseases and arthritis. Additionally, it is a frequent cell culture contaminant and has recently been detected in human gastric carcinoma tissues (13). *M. hyorhinis* MCLD has been isolated from a primary human melanoma cell line (5). Although mycoplasmas were considered adherent extracellular microorganisms (10), it has been shown that *M. hyorhinis* MCLD invades host cells (7). Furthermore, host cells infected with *M. hyorhinis* MCLD showed elevated expression of a CD99 ligand on melanoma cells (5) and a marked increase in the cellular concentration of the protease inhibitor calpastatin within infected neuroblastoma cells (4).

The *M. hyorhinis* MCLD genome was sequenced by using GS FLX Titanium technology and annotated using RAST (1) and PGAAP with manual curation. The fully assembled circular chromosome has 829,709 bp and an average G+C content of 25.9%. We predicted 778 coding sequences (CDSs) with a coding density of 89.2%, of which 273 CDSs were hypothetical or conserved hypothetical proteins.

Genome alignment of *M. hyorhinis* MCLD with the *M. hyorhinis* HUB-1 sequence (8), starting at the *dnaA* gene (SRH_2175) in the reverse complement orientation and using MAUVE (3), revealed 18 locally colinear blocks (LCBs). Unlike recent data on *M. mycoides* (12), no function-specific LCBs were identified. The MAUVE progressive mode utilized to identify conserved large segments revealed that each chromosome was composed of 4 regions and that differences may be explained by 2 inversions (14.4 kb, SRH_02645 to SRH_02545, and 3.7 kb, SRH_2605 to SRH_2635).

Similar to other mycoplasmas, 10 putative transcriptional regulators were detected in the *M. hyorhinis* MCLD genome, among them two sigma factors and the transcriptional repressor HrcA. Unlike other mycoplasma species, no homologs of the RelA and SpoT families were found.

The bacterial translational system components are conserved and included 30 tRNAs and 21 tRNA synthetases, a single copy of the 16S-23S rRNA operon, and a separate 5S rRNA gene. Three protein initiation factors, 4 elongation factors, and a single peptide release factor (RF-1) were also identified. However, the peptide chain release factor 2 (RF-2) was not detected.

It has been proposed that the variable surface lipoprotein (*vlp*) locus in *M. hyorhinis* contains seven distinct *vlp* genes (2). Comparative analysis of the *vlp* locus among five *M. hyorhinis* strains (MCLD, GDL, SK76, HUB-1, and a clonal variant of SK76) revealed that the *vlp* genes in *M. hyorhinis* MCLD are reduced, containing only 4 genes, *vlpD* (SRH_00185), *vlpE* (SRH_00180), *vlpB* (SRH_00175), and *vlpC* (SRH_00170), with no IS elements within the *vlp* locus. The *vlp* locus of *M. hyorhinis* HUB-1, isolated from pneumonic swine, and the arthritogenic SK76 strain (14) contain seven *vlp* genes (*vlpA* to *vlpG*), and the cell-culture isolate *M. hyorhinis* GDL possesses six (*vlpA* to *vlpF*) *vlp* genes (2, 8, 15). Only three *vlp* genes (*vlpA* to *vlpC*) were detected in the clonal variant SK76 (derived from the SK76 strain after broth medium passage [14]). Similar to other mycoplasmas with variable surface protein machinery (6, 9, 11), a putative integrase recombinase (SRH_00140) was found 5.3 kb downstream from the *vlpC* gene.

Nucleotide sequence accession number. This genome sequence of M. hyorhinis was deposited with annotation at GenBank under accession number CP002669. The version described in this paper is the first version.

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