

Genome-Wide Analysis of the First Sequenced *Mycoplasma capricolum* subsp. *capripneumoniae* Strain M1601

Shengli Chen,^{*1} Huafang Hao,^{*1} Ping Zhao,^{*} François Thiaucourt,^{†,‡} Ying He,^{*} Pengcheng Gao,^{*} Han Guo,^{*} Wenheng Ji,^{*} Zhanhui Wang,^{*} Zhongxin Lu,^{*} Yuefeng Chu,^{*,2} and Yongsheng Liu^{*,2}

^{*}State Key Laboratory of Veterinary Etiological Biology, Lanzhou Veterinary Research Institute, Chinese Academy of Agricultural Sciences, Lanzhou, 730046, Gansu, People's Republic of China, [†]CIRAD, UMR CMAEE, F-34398 Montpellier, France [‡]INRA, UMR1309 CMAEE, F-34398 Montpellier, France

ORCID ID: 0000-0001-9043-0351 (S.C.)

ABSTRACT *Mycoplasma capricolum* subsp. *capripneumoniae* (Mccp) is a common pathogen of goats that causes contagious caprine pleuropneumonia. We closed the gap and corrected rRNA operons in the draft genome of Mccp M1601: a strain isolated from an infected goat in a farm in Gansu, China. The genome size of M1601 is 1,016,707 bp with a GC content of 23.67%. We identified 915 genes (occupying 90.27% of the genome), of which 713 are protein-coding genes (excluding 163 pseudogenes). No genomic islands and complete insertion sequences were found in the genome. Putative determinants associated with the organism's virulence were analyzed, and 26 genes (including one adhesion protein gene, two capsule synthesis gene clusters, two lipoproteins, hemolysin A, ClpB, and proteins involved in pyruvate metabolism and cation transport) were potential virulence factors. In addition, two transporter systems (ATP-binding cassette [ABC] transporters and phosphotransferase) and two secretion systems (Sec and signal recognition particle [SRP] pathways) were observed in the Mccp genome. Genome synteny analysis reveals a good collinear relationship between M1601 and Mccp type strain F38. Phylogenetic analysis based on 11 single-copy core genes of 31 *Mycoplasma* strains revealed good collinearity between M1601 and *Mycoplasma capricolum* subsp. *capricolum* (Mcc) and close relationship among *Mycoplasma mycoides* cluster strains. Our genome-wide analysis of Mccp M1601 provides helpful information on the pathogenic mechanisms and genetics of Mccp.

KEYWORDS

Mycoplasma capricolum subsp. *capripneumoniae*
genome
virulence factor
comparative
analysis
phylogenetic
analysis
Genome Report

Mycoplasma capricolum subsp. *capripneumoniae* (Mccp) is the causative agent for contagious caprine pleuropneumonia (CCPP), a major infectious disease characterized by high morbidity in goats and its ability to cause considerable economic losses in Africa and Asia. The pathological lesions of CCPP are localized exclusively in the lungs and

pleura, and the pathological changes consist of a pleuropneumonia, unilateral hepatization, pleuritis, and an accumulation of pleural fluid (OIE 2016). The disease is now threatening disease-free countries and has been listed by the World Organisation for Animal Health (OIE) (Nicholas and Churchward 2012).

Mccp belongs to the genus *Mycoplasma* under the class *Mollicutes*. Isolating Mccp requires a high level of expertise and special growth medium. In addition, the organism is very fastidious and has slow growth. Until now, only about half of countries, where clinical disease has been reported, had isolated the causative organism (Manso-Silvan *et al.* 2011).

In 2007, a severe contagious respiratory disease occurred in a goat farm in the Gansu Province of China that has spread to other provinces. The main symptoms of this disease were coughing and high fever, which led to a morbidity rate of 62% and a mortality rate of 45%. The clinical symptoms and pathological changes were similar to CCPP. The organism was isolated from lungs of sick goats and placed in improved Thiaucourt's medium, purified three times, and named M1601. Biochemistry tests, 16S RNA sequence analysis, and animal pathogenicity tests were performed to further confirm the Mccp strain (Guo *et al.* 2011).

Copyright © 2017 Chen *et al.*

doi: <https://doi.org/10.1534/g3.117.300085>

Manuscript received June 8, 2017; accepted for publication July 19, 2017; published Early Online July 27, 2017.

This is an open-access article distributed under the terms of the Creative Commons Attribution 4.0 International License (<http://creativecommons.org/licenses/by/4.0/>), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

Supplemental material is available online at www.g3journal.org/lookup/suppl/doi:10.1534/g3.117.300085/-/DC1.

¹These authors contributed equally to this work.

²Corresponding authors: State Key Laboratory of Veterinary Etiological Biology, Lanzhou Veterinary Research Institute, Chinese Academy of Agricultural Sciences, Xujiaping 1, Lanzhou, 730046, Gansu, People's Republic of China. E-mails: chuyuefeng@caas.cn; and liyongsheng@caas.cn

In 2011, the first draft genome of Mccp strain M1601 was released (Chu *et al.* 2011). The genome of four other Mccp strains was reported later (Dupuy and Thiaucourt 2014; Falquet *et al.* 2014), but virulence factors of this important pathogen are still poorly understood. In this study, the gap of M1601 draft genome was closed, and the rRNA operon sequences were corrected to yield complete genomic sequences. Comprehensive genomic analysis of this pathogen was conducted. Putative determinants associated with Mccp virulence were identified based on the comprehensive genome analysis. Finally, comparative and phylogenetic analyses were performed. Understanding the supposed virulence genes, genome features, and genetics of this strain would be valuable in determining its pathogenic mechanisms and genetics.

MATERIALS AND METHODS

Bacterial growth and DNA extraction

Mccp M1601 was grown in improved Thiaucourt's medium (PPLO broth 21 g/liter, glucose 2 g/liter, sodium pyruvate 2 g/liter, 20% horse serum, 25% yeast extract 100 ml/liter, 0.4% phenol red 5 ml/liter, 100 units of penicillin, and 0.01% acetic acid thallium) at 37°. The 100 ml mid-log phase culture was harvested by centrifugation at 12,000 × *g* for 30 min and resuspended in 10 ml PBS (0.01 M, pH 7.2). Subsequently, total genomic DNA was extracted with a TIANamp Bacteria DNA Kit (Tiangen, Beijing, China) according to manufacturer's instructions.

Gap closure and rRNA operon sequences correction

The M1601 draft genome sequence with one gap cited in 760,982–761,498 was previously described (Chu *et al.* 2011). Gap closure was conducted as follows: The corresponding sequence was extracted from Mccp Abomsa-9231 sequence (NZ_LM995445) and used as a reference template for assembly. The assembly was then corrected manually. The gap consisted of 517 bp, and it was then inserted into the M1601 draft genome. There are two sets of rRNA operon sequence in the genome, one operon of M1601 strain was PCR amplified and sequenced by Sanger method. Another operon sequence was deduced after performing an assembly with the previous operon, being chosen as the reference sequence. The corrected rRNA operon sequences were then replaced in the corresponding M1601 sequence, yielding complete genomic sequences (GenBank under accession number NZ_CP017125).

Annotation and sequence analysis

The complete sequence was analyzed using Glimmer 3.0 (Delcher *et al.* 1999) for open reading frames containing >30 predicted amino acid residues. Transfer RNA (tRNA) and ribosomal RNA (rRNA) genes were predicted using tRNAscan-SE (Lowe and Eddy 1997) and Aragorn (Laslett and Canback 2004), and RNAmmer (Lagesen *et al.* 2007), respectively. Insertion and deletion (InDel) detection was conducted using LASTZ software (Harris 2007) to compare M1601 with *Mycoplasma capricolum* subsp. *capricolum* (Mcc) reference strain 27343. The best match results (<10 bp) were then extracted by using axtBest to obtain the preliminary InDel results. The 150 bp (3 × SD) from upstream and downstream of the reference sequence InDel sites were aligned and validated with the sample sequencing reads by BWA software (Li and Durbin 2009). After filtering, the reliable InDel sites were obtained. The genomic islands and insertion sequences were found by using Path-DIOMB (Hsiao *et al.* 2003) and ISfinder (<https://www-is.biotoul.fr/>), respectively.

The function annotation of the predicted protein-coding genes was conducted by blasting based on the COG, KEGG, Swiss-Prot, TrEMBL,

and NCBI-NR databases. Pseudogenes were detected by BLASTN analysis, and then the annotation was revised manually.

The putative virulence genes were identified by gene annotation and reference studies (O'Riordan *et al.* 2003; Chastanet *et al.* 2004; Hames *et al.* 2009; Bürki *et al.* 2015; Gründel *et al.* 2015). BLASTP searches (*E*-value <1e−5) against the NCBI database were applied, and the results were filtered by selecting the highest score of alignment (homology identity >40% and minimal alignment length percentage >40%). Core genes and specific genes were analyzed by CD-HIT software (Li and Godzik 2006) for clustering similar proteins with a threshold of 50% pairwise identity and 0.7 length difference cutoff in amino acids.

Comparative and phylogenetic analysis

Genomic alignment of Mccp strains M1601 and F38 was conducted using MUMmer (Delcher *et al.* 2003) and LASTZ (Harris 2007). Genomic synteny was performed based on the alignment results. Multiple sequence alignments of single-copy of core genes among 31 *Mycoplasma* strains were performed using MUSCLE (Edgar 2004). The phylogenetic tree was constructed by TreeBeST (Nandi *et al.* 2010) using the maximum likelihood method with 1000 bootstrap replicates. The genome sequences of other *Mycoplasma* strains were downloaded from the NCBI database.

Data availability

The genome sequence data were deposited in GenBank with the accession number NZ_CP017125. Supplemental Material, Figure S1 shows a comparison of genomic structure between Mccp strain M1601 and Mcc ATCC 27343. Figure S2 shows a comparison of complete genome between Mccp strains M1601 and F38. Table S1 shows an overview of the predicted results of the Mccp M1601 genome. Table S2 shows the genes involved in transport and metabolism. Table S3 shows the transporter system of Mccp. Table S4 shows the proteins involved in the secretion system. Table S5 shows the predicted genes involved in DNA replication. Table S6 shows the predicted genes involved in transcription. Table S7 shows the predicted genes involved in translation. Table S8 shows the InDel analysis between the M1601 genome and reference strain sequence. Table S9 shows the genome information of Mccp strain M1601 and four other partially annotated Mccp strains.

RESULTS

Genome features

The Mccp strain M1601 genome contains a single, circular chromosome of 1,016,707 bp with GC content of 23.67%, in line with the low GC content characteristics of *Mycoplasma* (Figure 1). A total of 915 genes were identified and occupy 90.27% of the genome. The genome contains 713 protein-coding genes (excluding 163 pseudogenes), six rRNA genes, 30 tRNA genes, and three ncRNA genes (Table S1). Among the protein-coding genes, 461 genes (50.38%) were assigned into specific functional clusters of orthologous groups families, comprising 21 functional categories (Table 1). No genomic islands and complete insertion sequences were detected in the genome. The genome sequence data were deposited in GenBank with the accession number NZ_CP017125.

Virulence factors

Adhesion is the first step of *Mycoplasma* infection of host cells; thus, adhesion proteins can be regarded as virulence-associated proteins of the pathogen (Razin *et al.* 1998). One adhesion-related gene (XDU01000267) was found in the Mccp M1601 genome. The capsule is often thought to be an important virulence factor for some pathogenic bacterium, such as *Pasteurella multocida* (Boyce and Adler 2000)

■ **Table 1 Functional category in COG of Mccp**

Code	Functional category	F38	M1601	Common
C	Energy production and conversion	25	25	25
D	Cell cycle control, cell division, chromosome partitioning	5	5	5
E	Amino acid transport and metabolism	32	30	30
F	Nucleotide transport and metabolism	29	29	29
G	Carbohydrate transport and metabolism	42	41	41
H	Coenzyme transport and metabolism	22	20	20
I	Lipid transport and metabolism	13	13	13
J	Translation, ribosomal structure and biogenesis	139	138	138
K	Transcription	26	26	26
L	Replication, recombination and repair	43	44	43
M	Cell wall/membrane/envelope biogenesis	8	8	8
N	Cell motility	2	2	2
O	Posttranslational modification, protein turnover, chaperones	18	18	18
P	Inorganic ion transport and metabolism	21	22	21
Q	Secondary metabolites biosynthesis, transport and catabolism	2	2	2
R	General function prediction only	34	32	32
S	Function unknown	8	9	8
T	Signal transduction mechanisms	13	13	13
U	Intracellular trafficking, secretion, and vesicular transport	5	5	5
V	Defense mechanisms	10	10	10
X	Mobilome: prophages, transposons	3	3	3
—	Total in COG	465	461	458

are involved in potassium and sodium uptake, respectively. These proteins may be associated with the virulence of Mccp.

Transporter, metabolism, and secretion

The biosynthetic capacity of *Mycoplasma* is severely poor, and most nutrition is obtained from the host during the intracellular lifestyle (Roger and Robin 1998). Thus, many genes are involved in *Mycoplasma* transporter and metabolism systems. In the M1601 genome, 175 genes were identified, which were related to transporter and metabolism systems (Table S2). In total, 164 genes were involved in amino acid, nucleotide, carbohydrate, inorganic ion, and coenzyme transport and metabolism, whereas 11 other genes were related with lipid and secondary metabolite biosynthesis, transport, and catabolism. Two transporter systems, ABC transporter system and the phosphotransferase system (PTS), were identified. Forty-eight genes encode the ABC-type transporter systems including 22 ABC transporter ATP-binding proteins, 22 ABC transporter permeases, two ATPase components, and two other proteins. By contrast, 18 genes encode for the PTS transport system (Table S3).

Protein secretion systems are also important for *Mycoplasma* survival in the host. The Mccp encodes nine proteins that are involved in protein secretion systems, including the Sec and SRP pathways (Table S4). The Sec system contains six proteins, SecA, SecD, SecE, SecG, SecY, and YidC. SRP-docking proteins FtsY and ffh were identified to participate in the SRP pathway. In addition, one lipoprotein signal peptidase A8 (XDU01000432) was found, whereas the signal peptidase I gene was absent.

Replication, transcription, and translation

In the Mccp genome, *dnaA* encoded by XDU01000001 binds to the DnaA box as an ATP-bound complex at the origin of replication, during the initiation of chromosomal replication. Fifty genes, which encode DNA polymerase III, DNA helicase, DNA polymerase I, 5'-3' exonuclease, endonuclease, repair protein, and NAD-dependent DNA ligase, were found to be involved in replication, recombination, and repair (Table S5).

In total, 27 genes (Table S6) were involved in transcription whereas 108 genes (Table S7) were related to translation, ribosomal structure, and biogenesis. Transcription elongation and termination were regulated by NusA, NusB, NusG, and GreA. GreA is a transcription elongation factor which could prevent transcription arrest, and NusA can induce transcription pausing, or stimulate anti-termination together with NusB and NusG (Borukhov *et al.* 2005). Eight transcriptional regulators were found in the Mccp genome, which include two RpiR and DeoR, one ROK, GntR, HrcA, and Fur (Table S6). In addition, 50 ribosomal proteins, 21 tRNA synthetase genes, and 11 translation factors were found in the genome (Table S7).

Comparative and evolutionary analysis

To reveal the genetic relationship between Mccp and Mcc, the Mccp strain M1601 genome and Mcc reference strain ATCC27343 (NC_007633) were compared (Figure S1). The size of the Mcc ATCC 27343 genome was 6684 bp shorter than the Mccp M1601 genome. For 870 genes in the Mcc ATCC 27343, 79.43% of them are observed in the Mccp genome. No large-scale insertion, deletions, and inversions existed between these two genomes, whilst there were 416 insertion/deletions found, these included 196 (47.12%) insertions and 220 (52.88%) deletions (Table S8). The genome comparisons among the sequenced Mccp strain M1601 and four other annotated Mccp strains were also conducted and are listed in Table S9.

In addition, the synteny of M1601 and the reference genome Mccp F38 was analyzed. Three blocks were developed with 99.86% identity. The block type exhibited forward collinearity, non-translocation, and non-inversion. Relative to the F38 genome, 870 bp deficiency and 446 bp insertion existed between the first two blocks and the last two blocks in M1601. Therefore, a good collinear relationship exists between M1601 and F38 (Figure S2).

A phylogenetic tree based on 11 single-copy core genes of 31 *Mycoplasma* strains was constructed (Figure 2). The results indicated a close relationship between Mccp M1601 and four other strains. All strains of Mccp, *Mycoplasma mycoides* subsp. *mycoides* SC (MmmSC),

Table 2 Potential virulence factors involved in the M1601 genome

Locus	Product	Gene	Gene length (bp)	Site	Prevalence in other four Mccp genome ^a	Prevalence in mycoplasma strain ^b
XDU01000037	P60 surface lipoprotein	p60	1644	46190..47833	100% (4/4)	30.8% (8/26)
XDU01000067	Hemolysin A	—	729	84200..84928	100% (4/4)	57.7% (15/26)
XDU01000075	Glycosyl transferase	—	1575	90225..91799	100% (4/4)	84.6% (22/26)
XDU01000076	UTP-glucose-1-phosphate uridylyltransferase	galU	879	91802..92680	100% (4/4)	57.7% (15/26)
XDU01000099	Magnesium-translocating P-type ATPase	mgfA	2829	117833..120661	100% (4/4)	50.0% (13/26)
XDU01000242	Glycerol facilitator factor	glpF	780	288715..289494	100% (4/4)	50.0% (13/26)
XDU01000243	Glycerol kinase	glpK	1149	289530..290678	100% (4/4)	73.1% (19/26)
XDU01000244	Glycerol-3-phosphate dehydrogenase	glpD	1164	291065..292228	100% (4/4)	88.5% (23/26)
XDU01000249	Lipoate-protein ligase A	lplA	1005	299248..300252	100% (4/4)	65.4% (17/26)
XDU01000251	Pyruvate dehydrogenase E1 component subunit beta	pdhB	990	301399..302388	100% (4/4)	80.8% (21/26)
XDU01000252	Branched-chain alpha-keto acid dehydrogenase subunit E2	pdhC	1317	302417..303733	100% (4/4)	50.0% (13/26)
XDU01000253	Dihydrolipoamide dehydrogenase (E3) component	pdhD	1890	303753..305642	100% (4/4)	84.6% (22/26)
XDU01000267	Adhesion-related protein	—	717	321984..322700	100% (4/4)	26.9% (7/26)
XDU01000405	Chaperone protein ClpB	clpB	2142	474287..476428	100% (4/4)	76.9% (20/26)
XDU01000466	Dihydrolipoamide dehydrogenase (E3) component	pdhD	1362	535434..536795	100% (4/4)	57.7% (15/26)
XDU01000483	Lipoate-protein ligase A	lplA	1038	552430..553467	100% (4/4)	46.2% (12/26)
XDU01000486	ABC transporter permease	gtsC	804	557034..557837	100% (4/4)	46.2% (12/26)
XDU01000487	Glycerol ABC transporter permease	gtsB	1005	557815..558819	100% (4/4)	46.2% (12/26)
XDU01000488	ABC transporter ATP-binding protein	gtsA	1212	558812..560023	100% (4/4)	53.8% (14/26)
XDU01000612	Lipoproteins VmcC	—	399	699233..699631	100% (4/4)	23.1% (6/26)
XDU01000742	Sodium transporter	—	1602	828031..829632	100% (4/4)	30.8% (8/26)
XDU01000743	Potassium transporter TrkA	trkA	729	829711..830439	100% (4/4)	30.8% (8/26)
XDU01000796	Magnesium transporter	mgfE	1404	884553..885956	100% (4/4)	73.1% (19/26)
XDU01000814	Diacylglycerol transferase	—	1581	907200..908780	100% (4/4)	57.7% (15/26)
XDU01000816	Diacylglycerol transferase	—	1431	909705..911135	100% (4/4)	69.2% (18/26)
XDU01000848	Magnesium transporter	mgfA	1116	944515..945630	100% (4/4)	30.8% (8/26)

^aMccp strains including F38 (NZ_LN15398), ILR181(NZ_LN15399), 87001(NZ_CP006959), and 9231(NZ_LMP95445).

^bTwenty-six other species of Mycoplasma strains for which genome is used for the phylogenetic analysis except the five Mccp strains.

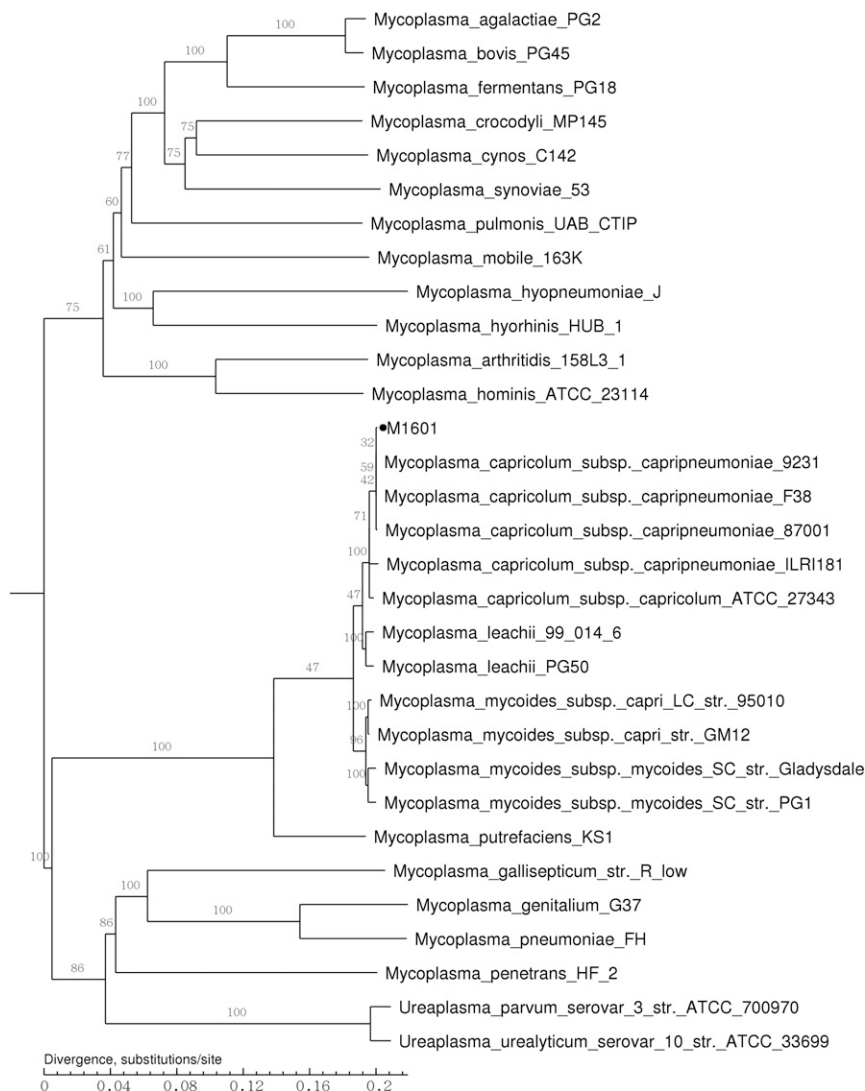


Figure 2 Phylogenetic tree based on 11 single-copy core genes of 31 selected *Mycoplasma*. The phylogenetic tree was constructed by TreeBeST using the maximum likelihood method with 1000 bootstrap replicates. The bootstrap numbers are given for each node. The tree is drawn to scale, with branch lengths measured in the number of substitutions per site. The Mccp strain M1601 is highlighted by black circles.

M. mycoides subsp. *capri* (Mmc), Mcc, and *M. leachii* (Ml), belong to a large *M. mycoides* cluster.

DISCUSSION

Adhering to host cell is a crucial step in the process of *Mycoplasma* infection and colonization, and is an important aspect of the research of pathogenic mechanisms. At present, several adhesion proteins of various *Mycoplasma* species have been identified, such as variable surface lipoproteins (Sachse *et al.* 2000), α -enolase (Song *et al.* 2012), VpmaX protein (Zou *et al.* 2013) of *M. bovis*, P50 of *M. hominis* (Kitzerow *et al.* 1999), LppS of *M. conjunctivae* (Belloy *et al.* 2013), and P19 of *M. mycoides* subsp. *mycoides* (Mmm) (Zhou *et al.* 2016). A previous study reported that a hypothetical membrane protein encoded by the 0297 gene of Mccp strain C87001 showed significant adhesion on goat bronchial epithelial cells (Bai *et al.* 2014). The XDU01000267 gene, corresponding to homologous 0297 gene, may be an adhesion protein of Mccp, but this finding needs further verification.

Vsps play an important role in *Mycoplasma* colonization and adaptation to the host environment in different infection stages. Vsps are also related to antigenicity and immune regulation of *Mycoplasma* (Buchenau *et al.* 2010; Bolland and Dybvig 2012). Variable surface

lipoprotein gene cluster exists in many *Mycoplasma* genomes, such as Mmc, Mcc, and MmmSC. In M1601, one VmcC and one P60 surface lipoprotein were found, and both were considered to be potential virulence factors. In addition, proteins related to capsule synthesis and pyruvate metabolism were also related with bacterial virulence (Boyce and Adler 2000; O’Riordan *et al.* 2003; Gates *et al.* 2008; Gründel *et al.* 2015). We found two gene clusters involved in capsule synthesis and six pyruvate-metabolism-related enzyme genes in the Mccp genome, and all these genes may be associated with Mccp virulence.

At present, seven types of protein secretion systems in bacteria have been identified (Abdallah *et al.* 2007). A signal peptide present at N-terminal on the secreted protein via Sec pathway is required and cleaved to the mature form (Beckwith 2013). Signal peptidases are proteases that remove the N-terminal signal peptide of secreted proteins in the endoplasmic reticulum. Signal peptidase I gene and lipoprotein signal peptidase gene are found in *Mycoplasma* species such as *M. conjunctivae* (Calderon-Copete *et al.* 2009), *M. hyopneumoniae* (Moitinho-Silva *et al.* 2012), *M. pulmonis* (Chambaud *et al.* 2001), and *M. synoviae* (Vasconcelos *et al.* 2005). However, in the Mccp M1601 genome, only one lipoprotein signal peptidase gene was found, which was similar to *M. bovis* (Li *et al.* 2011). This finding indicated

that Mccp may have the same mechanism of extracellular protein secretion as *M. bovis* but different from *M. pulmonis*, *M. hyopneumoniae*, and *M. synoviae*.

DNA replication, recombination, and repair in the Mccp genome were also analyzed, and 50 proteins were involved in these biological processes. However, no typical mismatch-repair system (MutHLS) genes were found. The error may be repaired mainly by RecF pathway which including recombinational repair, the nucleotide excision repair system and the base excision repair system as previously reported (Carvalho *et al.* 2005).

M1601 and Mcc strain ATCC 27343 kept good collinearity, suggesting closer genetic relationship between Mccp and Mcc. Good collinear relationship was seen for Mccp strains M1601 and F38. The phylogenetic analysis indicated that Mccp, Mmc, Mcc, Mmm, and Ml all belong to the Mm cluster, which is in accordance with the result of collinearity analysis and morphological and biochemical features.

ACKNOWLEDGMENTS

We thank François Thiaucourt and Julien Gerard at CIRAD, France, for their help in the gap closure and the correction of rRNA operon sequences to obtain the complete genome sequence of M1601. This work was funded by the National Key Technologies R&D Program (2015BAD12B02), the National Key Research and Development Plan (2016YFD0500907), the Fundamental Research Funds for CAAS (Y2016CG24, Y2016PT42), and the State Key Laboratory of Veterinary Etiological Biology through the Key Hatch Project for *Mycoplasma* Research of Y.C.

Author contributions: Conceived and designed the experiments: Y.C. and Y.L. Performed the experiments: S.C. and H.H. Analyzed the data: S.C., H.H., Y.C., P.Z., and Y.H. Contributed reagents/materials/analysis tools: P.G., H.G., W.J., Z.W., and Z.L. Wrote the paper: S.C., H.H., and Y.C. All authors read and approved the final manuscript. The authors declare that they have no competing interests. The founding sponsors had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript; and in the decision to publish the results.

LITERATURE CITED

- Abdallah, A. M., N. C. Gey van Pittius, P. A. Champion, J. Cox, and J. Luijck, 2007 Type VII secretion-mycobacteria show the way. *Nat. Rev. Microbiol.* 5: 883–891.
- Bai, F., Y. Li, J. Wu, Y. Wang, S. Liu *et al.*, 2014 Characterization of the adhesion-related protein in *Mycoplasma capricolum* subspecies *Capripneumoniae*. *Chin. Vet. Sci.* 44: 583–588.
- Beckwith, J., 2013 The sec-dependent pathway. *Res. Microbiol.* 164: 497–504.
- Belloy, L., E. M. Vilei, M. Giacometti, and J. Frey, 2013 Characterization of LppS, an adhesin of *Mycoplasma conjunctivae*. *Microbiology* 149: 185–193.
- Bolland, J. R., and K. Dybvig, 2012 *Mycoplasma pulmonis* Vsa proteins and polysaccharide modulate adherence to pulmonary epithelial cells. *FEMS Microbiol. Lett.* 331: 25–30.
- Borukhov, S., J. Lee, and O. Laptchenko, 2005 Bacterial transcription elongation factors: new insights into molecular mechanism of action. *Mol. Microbiol.* 55: 1315–1324.
- Boyce, J. D., and B. Adler, 2000 The capsule is a virulence determinant in the pathogenesis of *Pasteurella multocida* M1404 (B:2). *Infect. Immun.* 68: 3463–3468.
- Buchenau, I., F. Poumarat, D. Le Grand, H. Linkner, R. Rosengarten *et al.*, 2010 Expression of *Mycoplasma bovis* variable surface membrane proteins in the respiratory tract of calves after experimental infection with a clonal variant of *Mycoplasma bovis* type strain PG45. *Res. Vet. Sci.* 89: 223–229.
- Bürki, S., J. Frey, and P. Pilo, 2015 Virulence, persistence and dissemination of *Mycoplasma bovis*. *Vet. Microbiol.* 179: 15–22.
- Calderon-Copete, S. P., G. Wigger, C. Wunderlin, T. Schmidheini, J. Frey *et al.*, 2009 The *Mycoplasma conjunctivae* genome sequencing, annotation and analysis. *BMC Bioinformatics* 10: S7.
- Carvalho, F. M., M. M. Fonseca, S. Batistuzzo De Medeiros, K. C. Scortecci, C. A. Blaha *et al.*, 2005 DNA repair in reduced genome: the *Mycoplasma* model. *Gene* 360: 111–119.
- Chambaud, I., R. Heilig, S. Ferris, V. Barbe, D. Samson *et al.*, 2001 The complete genome sequence of the murine respiratory pathogen *Mycoplasma pulmonis*. *Nucleic Acids Res.* 29: 2145–2153.
- Chastanet, A., I. Derre, S. Nair, and T. Msadek, 2004 clpB, a novel member of the *Listeria monocytogenes* CtsR regulon, is involved in virulence but not in general stress tolerance. *J. Bacteriol.* 186: 1165–1174.
- Chu, Y., P. Gao, P. Zhao, Y. He, N. Liao *et al.*, 2011 Genome sequence of *Mycoplasma capricolum* subsp. *capripneumoniae* strain M1601. *J. Bacteriol.* 193: 6098–6099.
- Delcher, A. L., D. Harmon, S. Kasif, O. White, and S. L. Salzberg, 1999 Improved microbial gene identification with GLIMMER. *Nucleic Acids Res.* 27: 4636–4641.
- Delcher, A. L., S. L. Salzberg, and A. M. Phillippy, 2003 Using MUMmer to identify similar regions in large sequence sets. *Curr. Protoc. Bioinformatics* 10: 3.1–3.18.
- Dupuy, V., and F. Thiaucourt, 2014 Complete genome sequence of *Mycoplasma capricolum* subsp. *capripneumoniae* strain 9231-Abomsa. *Genome Announc.* 2: e01067–14.
- Edgar, R. C., 2004 MUSCLE: a multiple sequence alignment method with reduced time and space complexity. *BMC Bioinformatics* 5: 113.
- Falquet, L., A. Liljander, E. Schieck, I. Gluecks, and J. Frey, 2014 Complete genome sequences of virulent *Mycoplasma capricolum* subsp. *capripneumoniae* strains F38 and ILRI181. *Genome Announc.* 2: e01041–14.
- Gates, A. E., S. Frasca, A. Nyaoke, T. S. Gorton, L. K. Silbart *et al.*, 2008 Comparative assessment of a metabolically attenuated *Mycoplasma gallisepticum* mutant as a live vaccine for the prevention of avian respiratory mycoplasmosis. *Vaccine* 26: 2010–2019.
- Goebel, W., T. Chakraborty, and J. Kreft, 1988 Bacterial hemolysins as virulence factors. *Antonie van Leeuwenhoek* 54: 453–463.
- Groisman, E. A., K. Hollands, M. A. Kriner, E. J. Lee, S. Y. Park *et al.*, 2013 Bacterial Mg²⁺ homeostasis, transport, and virulence. *Annu. Rev. Genet.* 47: 625–646.
- Gründel, A., K. Friedrich, M. Pfeiffer, E. Jacobs, and R. Dumke, 2015 Subunits of the pyruvate dehydrogenase cluster of *Mycoplasma pneumoniae* are surface-displayed proteins that bind and activate human plasminogen. *PLoS One* 10: e0126600.
- Guo, H., Y. Chu, P. Zhao, P. Gao, Y. He *et al.*, 2011 Isolation and identification of *Mycoplasma capricolum* subsp. *capripneumoniae* in Gansu province. *Chin. J. Vet. Sci.* 31: 352–356.
- Hames, C., S. Halbedel, M. Hoppert, J. Frey, and J. Stülke, 2009 Glycerol metabolism is important for cytotoxicity of *Mycoplasma pneumoniae*. *J. Bacteriol.* 191: 747–753.
- Harris, R. S., 2007 *Improved Pairwise Alignment of Genomic DNA*. ProQuest, Ann Arbor, MI.
- Hsiao, W., I. Wan, S. J. Jones, and F. S. Brinkman, 2003 IslandPath: aiding detection of genomic islands in prokaryotes. *Bioinformatics* 9: 418–420.
- Kitzerow, A., U. Hadding, and B. Henrich, 1999 Cyto-adherence studies of the adhesin P50 of *Mycoplasma hominis*. *J. Med. Microbiol.* 48: 485–493.
- Lagesen, K., P. Hallin, E. A. Rødland, H. H. Staerfeldt, T. Rognes *et al.*, 2007 RNAmmer: consistent and rapid annotation of ribosomal RNA genes. *Nucleic Acids Res.* 35: 3100–3108.
- Laslett, D., and B. Canback, 2004 ARAGORN, a program to detect tRNA genes and tmRNA genes in nucleotide sequences. *Nucleic Acids Res.* 32: 11–16.
- Li, H., and R. Durbin, 2009 Fast and accurate short read alignment with Burrows-Wheeler transform. *Bioinformatics* 25: 1754–1760.
- Li, W., and A. Godzik, 2006 Cd-hit: a fast program for clustering and comparing large sets of protein or nucleotide sequences. *Bioinformatics* 22: 1658–1659.

- Li, Y., H. Zheng, Y. Liu, Y. Jiang, J. Xin *et al.*, 2011 The complete genome sequence of *Mycoplasma bovis* strain Hubei-1. *PLoS One* 6: e20999.
- Lowe, T. M., and S. R. Eddy, 1997 tRNAscan-SE: a program for improved detection of transfer RNA genes in genomic sequence. *Nucleic Acids Res.* 25: 955–964.
- Manso-Silvan, L., V. Dupuy, Y. Chu, and F. Thiaucourt, 2011 Multi-locus sequence analysis of *Mycoplasma capricolum* subsp. *capripneumoniae* for the molecular epidemiology of contagious caprine pleuropneumonia. *Vet. Res.* 42: 86.
- March, J. B., and M. Brodli, 2000 Comparison of the virulence of European and African isolates of *Mycoplasma mycoides* subspecies *mycoides* small colony type. *Vet. Rec.* 147: 20–21.
- Merino, S., R. Gavın, M. Altarriba, L. Izquierdo, M. E. Maguire *et al.*, 2001 The MgtE Mg²⁺ transport protein is involved in *Aeromonas hydrophila* adherence. *FEMS Microbiol. Lett.* 198: 189–195.
- Minato, Y., A. Ghosh, W. J. Faulkner, E. J. Lind, S. Schesser Bartra *et al.*, 2013 Na⁺/H⁺ antiport is essential for *Yersinia pestis* virulence. *Infect. Immun.* 81: 3163–3172.
- Moitinho-Silva, L., B. L. Heineck, L. A. Reolon, J. A. Paes, C. S. Klein *et al.*, 2012 *Mycoplasma hyopneumoniae* type I signal peptidase: expression and evaluation of its diagnostic potential. *Vet. Microbiol.* 154: 282–291.
- Nair, S., E. Milohanic, and P. Berche, 2000 ClpC ATPase is required for cell adhesion and invasion of *Listeria monocytogenes*. *Infect. Immun.* 68: 7061–7068.
- Nandi, T., C. Ong, A. P. Singh, J. Boddey, T. Atkins *et al.*, 2010 A genomic survey of positive selection in *Burkholderia pseudomallei* provides insights into the evolution of accidental virulence. *PLoS Pathog.* 6: e1000845.
- Nicholas, R., and C. Churchward, 2012 Contagious caprine pleuropneumonia: new aspects of an old disease. *Transbound. Emerg. Dis.* 59: 189–196.
- OIE. Available at: http://www.oie.int/fileadmin/Home/eng/Health_standards/tahm/2.07.05_CCPP.pdf. Accessed: June 28, 2016.
- O’Riordan, M., M. A. Moors, and D. A. Portnoy, 2003 *Listeria* intracellular growth and virulence require host-derived lipoic acid. *Science* 302: 462–464.
- Patel, M. S., N. S. Nemeria, W. Furey, and F. Jordan, 2014 The pyruvate dehydrogenase complexes: structure-based function and regulation. *J. Biol. Chem.* 289: 16615–16623.
- Pilo, P., J. Frey, and E. M. Vilei, 2007 Molecular mechanisms of pathogenicity of *Mycoplasma mycoides* subsp. *mycoides* SC. *Vet. J.* 174: 513–521.
- Razin, S., D. Yogeve, and Y. Naot, 1998 Molecular biology and pathogenicity of mycoplasmas. *Microbiol. Mol. Biol. Rev.* 62: 1094–1156.
- Roger, M., and N. Robin, 1998 *Mycoplasma* protocols, pp. 1–5 in *Methods in Molecular Biology*, edited by Walker, J. M. Humana Press, Clifton, NJ.
- Sachse, K., J. H. Helbig, I. Lysnyansky, C. Grajetzki, W. Muller *et al.*, 2000 Epitope mapping of immunogenic and adhesive structures in repetitive domains of *Mycoplasma bovis* variable surface lipoproteins. *Infect. Immun.* 68: 680–687.
- Seymour, L. M., C. Jenkins, A. T. Deutscher, B. B. Raymond, M. P. Padula *et al.*, 2012 Mhp182 (P102) binds fibronectin and contributes to the recruitment of plasmin(ogen) to the *Mycoplasma hyopneumoniae* cell surface. *Cell. Microbiol.* 14: 81–94.
- Song, Z., Y. Li, Y. Liu, J. Xin, X. Zou *et al.*, 2012 α -Enolase, an adhesion-related factor of *Mycoplasma bovis*. *PLoS One* 7: e38836.
- Su, J., H. Gong, J. Lai, A. Main, and S. Lu, 2009 The potassium transporter Trk and external potassium modulate *Salmonella enterica* protein secretion and virulence. *Infect. Immun.* 77: 667–675.
- Ueda, A., and T. K. Wood, 2008 Potassium and sodium transporters of *Pseudomonas aeruginosa* regulate virulence to barley. *Appl. Microbiol. Biotechnol.* 79: 843–858.
- Vasconcelos, A. T., H. B. Ferreira, C. V. Bizarro, S. L. Bonatto, M. O. Carvalho *et al.*, 2005 Swine and poultry pathogens: the complete genome sequences of two strains of *Mycoplasma hyopneumoniae* and a strain of *Mycoplasma synoviae*. *J. Bacteriol.* 187: 5568–5577.
- Vilei, E. M., and J. Frey, 2001 Genetic and biochemical characterization of glycerol uptake in *mycoplasma mycoides* subsp. *mycoides* SC: its impact on H(2)O(2) production and virulence. *Clin. Diagn. Lab. Immunol.* 8: 85–92.
- Wise, K. S., M. F. Foecking, K. Roske, Y. J. Lee, Y. M. Lee *et al.*, 2006 Distinctive repertoire of contingency genes conferring mutation-based phase variation and combinatorial expression of surface lipoproteins in *Mycoplasma capricolum* subsp. *capricolum* of the *Mycoplasma mycoides* phylogenetic cluster. *J. Bacteriol.* 188: 4926–4941.
- Zhou, Y., Y. Wang, Y. Li, N. Nick, X. Zou *et al.*, 2016 P19 contributes to *Mycoplasma mycoides* subsp. *mycoides* adhesion to EBL cells. *Microb. Pathog.* 93: 13–21.
- Zou, X., Y. Li, Y. Wang, Y. Zhou, Y. Liu *et al.*, 2013 Molecular cloning and characterization of a surface-localized adhesion protein in *Mycoplasma bovis* Hubei-1 strain. *PLoS One* 8: e69644.

Communicating editor: D-J. de Koning