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

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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-Silván 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).
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 Abomusa–9231 sequence (NZ_LM3955445) 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 sequences in the genome, one operon of M1601 strain was PCR amplified and sequenced by Sanger method. Another operon sequence was deducted 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.

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)

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and *Mycoplasma mycoides* subsp. *mycoides SC* (March and Brodie 2000; Piló et al. 2007). The genome contains a gene cluster (XDU01000075, XDU01000076, XDU01000814, and XDU01000816) involved in the synthesis of the capsule, comprising genes encoding glycosyltransferase, UTP–glucose-1-phosphate uridylyltransferase, and diacylglycerol transferase (Table 2).

ClpC is an ATPase that plays an important role in cell adhesion and invasion and is responsible for the virulence of *L. monocytogenes* (Nair et al. 2000). ClpB is a component of stress response in microorganisms and is responsible for the virulence of *Mycoplasma mycoides* (March and Brodlie 2000; Piló et al. 2007). ClpB is a component of stress response in microorganisms and is responsible for the virulence of *Mycoplasma mycoides* subsp. *capripneumoniae* (Chastanet et al. 2004). ClpB was also involved in the virulence of *L. monocytogenes* (Chastanet et al. 2004). Although clpC gene was not found, one clpB gene (XDU01000405) was identified in the genome of Mccp, and it shows 72% identity with the ClpB protein of *L. monocytogenes*. Thus, ClpB may be a virulence factor of Mccp.

Variable surface proteins (Vsps) have been thought to play an important role in the process of antigenic variation and immunity evasion, and are regarded to be a pathogenic factor for *Mycoplasma* (Bürki et al. 2015). VmcC is reported to play key role in the antigenic variation and survival of Mcc (Wise et al. 2006), and P60 surface lipoprotein is considered to be related to virulence of *M. hyopneumoniae* (Seymour et al. 2012). One VmcC lipoprotein (XDU01000612) and P60 surface lipoprotein (XDU01000037) were found in the genome. These identified lipoproteins may be associated with Mccp virulence.

Hemolysins are toxic proteins that cause the lysis of erythrocytes by forming pores in their membranes (Goebel et al. 1988). Hemolysin A (XDU01000067) was identified in the Mccp genome, and it could be considered as a virulence factor.

Pyruvate is the first product in the process of aerobic metabolism of glucose. It goes to the mitochondrion to produce acetyl-CoA under catalysis of pyruvate dehydrogenase (PDH) enzyme complex. Lipoate–protein ligase (LplA) and PDH complex (composed of PDH E1, lipoic acid acetyltransferase E2, and dihydrolipoamide dehydrogenase E3) play a critical role in pyruvate metabolism (Patel et al. 2014). A mutant of dihydrolipoamide dehydrogenase E3 was significantly attenuated in *M. gallisepticum in vivo* (Gates et al. 2008). Recent research indicated that pyruvate metabolism component PDH subunits may contribute to the pathogenesis of *M. pneumoniae* infections by interaction with human plasminogen (Gründel et al. 2015). LplA ligates lipoic acid from the host to the PDH E2 component to generate E2-lipoamide, which plays an important role in pyruvate metabolism. *L. monocytogenes* lacking LplA1 were defective for growth in the host cytosol and attenuated 300-fold compared with wild-type strain (O’Riordan et al. 2003). Four PDH complex genes and two lpla genes were identified in the genome, and they were regarded to be virulence factors (Table 2).

Glycerol metabolism and production of *H₂O₂* are considered to be associated with *Mycoplasma* virulence (Vilei and Frey 2001; Hames et al. 2009). The glpF–glpK–glpD (XDU01000242, XDU01000243, and XDU01000244) and gtsA–gtsB–gtsC gene clusters (XDU01000486, XDU01000487, and XDU01000488), which are involved in glycerol metabolism, were identified in the Mccp genome.

Magnesium transporters MgtA and MgtE have been shown to be related to virulence of some bacteria (Grosman et al. 2013), such as *Aeromonas hydrophila* (Merino et al. 2001). In the Mccp genome, three magnesium transporters genes (XDU01000099, XDU01000796, XDU01000848) were found. Potassium transporter TrkA is related to virulence of *Salmonella* (Su et al. 2009), and sodium transporter is reported to be associated with the virulence of *Yersinia pestis* (Minato et al. 2013) and *Pseudomonas aeruginosa* (Ueda and Wood 2008). One potassium transporter TrkA (XDU01000743) and one sodium transporter (XDU01000742) were found in the genome, which...
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 fflf 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),
| 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 | mgtA | 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         | lipA | 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         | lipA | 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            | mgtE | 1404             | 884553..885956 | 100% (4/4)                                  | 73.1% (19/26)                          |
| XDU01000814       | Dicacylglycerol transferase      | —    | 1581             | 907200..908780 | 100% (4/4)                                  | 57.7% (15/26)                          |
| XDU01000816       | Dicacylglycerol transferase      | —    | 1431             | 909705..911135 | 100% (4/4)                                  | 69.2% (18/26)                          |
| XDU01000848       | Magnesium transporter            | mgtA | 1116             | 944515..945630 | 100% (4/4)                                  | 30.8% (8/26)                           |

\(^a\)Mccp strains including F38 (NZ_LN515398), ILR1811(NZ_LN515399), 87001(NZ_CP006959), and 9231(NZ_LM995445).

\(^b\)Twenty-six other species of Mycoplasma strains for which genome is used for the phylogenetic analysis except the five Mccp strains.
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* (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 nucleoside 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 Mcp strains M1601 and F38. The phylogenetic analysis indicated that Mcp, 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.

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LITERATURE CITED
Abdallah, A. M., N. C. Gey van Pittius, P. A. Champion, J. Cox, and J. Luirink, 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 subsp. 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. Lapanke, 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 (B2). Infect. Immun. 68: 3463–3468.
Buchanen, I., F. Pounarat, 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 donal variant of Mycoplasma bovis type strain PG45. Res. Vet. Sci. 89: 223–229.
Bürgi, 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: 57.
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. Mseadek, 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: 6098–6109.
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. Phillips, 2003 Using MUMmer to identify similar regions in large genome sets. Curr. Protoc. Bioinformatics. 10: 3.1–3.18.
Dupuy, V., and F. Chasseaud, 2014 Complete genome sequence of Mycoplasma capricolum subsp. capripneumoniae strain 9231-Abomas. 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. Lijlder, E. Schiec, I. Gluecks, and J. Frey, 2014 Complete genome sequences of virulent Mycoplasma capricolum subsp. capripneumoniae strains F38 and ILR181. 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. Pleffer, 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. Pro-Quest, Ann Arbor, MI.
Hisao, 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. Redland, H. H. Stafellf, 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-Silván, 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. Brodlie, 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. Gavín, 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/pdf/1-07_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. Yoge, 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. Müller 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., 2012 Mycoplasma hyopneumoniae type I signal peptidase: expression and evaluation of its diagnostic potential. Vet. Microbiol. 154: 282–291.

Nicholas, R., and C. Churchward, 2012 Contagious caprine pleuropneumonia: new aspects of an old disease. Transbound. Emerg. Dis. 59: 189–196.