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Cloning and Characterization of a Putative Cytadhesin Gene (*mgc1*) from *Mycoplasma gallisepticum*†

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A 150-kDa cytadhesin-like protein from *Mycoplasma gallisepticum* has been identified. A previously described 583-bp fragment (J. E. Dohms, L. L. Hnatow, P. Whetzel, R. Morgan, and C. L. Keeler, Jr., Avian Dis. 37:380–388, 1993) was used to probe a genomic library of *M. gallisepticum* DNA. An 8.0-kb *Sac*I fragment was identified, cloned, and partially sequenced. Analysis of the resulting 3,750-bp sequence revealed the presence of a 3,366-nucleotide open reading frame, *mgc1*. The 1,122-amino-acid protein encoded by this open reading frame, MGC1, has characteristics of a class I membrane protein and has homology with the MgPa cytadhesin of *Mycoplasma genitalium* (26.3%) and the P1 cytadhesin of *Mycoplasma pneumoniae* (28.7%). A portion of MGC1 was expressed as a glutathione *S*-transferase fusion protein and used to produce antiserum in rabbits. The antiserum recognizes a 150-kDa protein from *M. gallisepticum*. The protein is sensitive to trypsin, confirming that it is surface exposed. Primer extension analysis indicates that the *mgc1* RNA starts within an upstream open reading frame, suggesting complex control of its expression. This is the first description of a functional gene from *M. gallisepticum* showing homology to cytadhesin genes from human mycoplasmas.

Members of the prokaryotic class *Mollicutes*, family *Mycoplasmataceae*, can colonize the respiratory tract of vertebrates, establish chronic infections, and cause disease. Although mycoplasmas possess the smallest recorded genomes of self-replicating organisms, like other pathogens, they use strategies that allow attachment to the host's mucosae in the face of innate and specific defense mechanisms. To colonize the respiratory tract, mycoplasmas must first penetrate a moving mucous sheet, using mechanisms thought to involve surface-dependent motility (5, 30, 41). Once attached, mycoplasma cells maintain a close association with the epithelial cell surface. By mechanisms that are not clearly understood, this relationship is needed for the bacterial cells to obtain essential nutrients.

Human, rodent, and avian mycoplasmas attach in vitro to a variety of cells, including ciliated epithelial cells (8, 15, 16, 24, 40), erythrocytes (2, 20, 35), spermatozoa (46), and fibroblasts (17–19). Mycoplasma-encoded cytadhesins mediate attachment to specific cells via sialylated glycoproteins or glycolipids (2, 3, 33). Cytadhesins of the human pathogens *Mycoplasma pneumoniae*, the causative agent of atypical pneumonia, and *Mycoplasma genitalium*, an agent implicated in nongonococcal urethritis, have been the subject of intensive study (41).

Mycoplasma gallisepticum is a significant respiratory pathogen of poultry, although infections may produce disease signs that vary in severity (49). Concurrent viral and bacterial infections produce a severe chronic respiratory disease characterized by high morbidity and mortality. In severe, uncomplicated infections, poultry flocks often show signs that include tracheal rales, nasal discharge, and coughing. At necropsy, sinusitis, pneumonia, and airsaculitis are commonly observed. *M. gallisepticum* infections of chicken and turkey reproductive tracts result in decreased egg production and permit vertical transmission to progeny (49).

M. gallisepticum, *M. pneumoniae*, and *M. genitalium* share structural and functional features involved in attachment to host tissues. All three mycoplasmas possess a terminal tip organelle that is involved in motility and is oriented toward the epithelium cell surface after attachment (24, 30). The major cytadhesin molecule of *M. pneumoniae*, P1, is an immunodominant 170-kDa protein found concentrated on the tip organelle (4, 14, 23–25). Western blot (immunoblot) analysis showed that antibodies directed against P1 cross-react with a 140-kDa *M. genitalium* adhesin, designated MgPa (7, 26, 39). It was further shown that antibodies directed against either P1 or MgPa cross-react with an *M. gallisepticum* protein variously reported to be 170 or 155 kDa (7, 9, 26). The *M. pneumoniae* and *M. genitalium* cytadhesin genes and their corresponding operons have been sequenced and characterized (10, 27–29, 44). Both the P1 and the MgPa genes are the second genes of three-gene operons. Significant homology also exists between the genes flanking the P1 and MgPa genes (28). Dallo and Baseman (9) used either P1 or MgPa DNA as probes in low-stringency Southern hybridizations to identify a related *M. gallisepticum* DNA fragment. Collectively, these findings suggest that there may be a conserved family of pathogenic mycoplasma cytadhesins that are utilized to colonize widely divergent hosts.

Recently, we identified a portion of the putative *M. gallisepticum* cytadhesin gene (13). Using degenerate oligonucleotide primers designed from conserved regions of the P1 and MgPa sequences, we amplified and sequenced a 583-bp *M. gallisepticum* DNA fragment, using the PCR. In this study, the work has been extended to include the complete sequence analysis and partial characterization of the putative *M. gallisepticum* cytadhesin gene.

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MATERIALS AND METHODS

Organisms and growth conditions. *M. gallisepticum* S6 was obtained from Y. M. Saif (Ohio Agricultural Research and Development Center, The Ohio State University, Wooster). Cultures were passaged in Frey broth medium according to previously described procedures (13). Broth stocks (100 ml), stored at −70°C, were used to inoculate 1 liter of Frey broth. Cultures were incubated at 37°C for 12 h and harvested during log phase for DNA isolation.

DNA isolation. *M. gallisepticum* cell pellets were washed with phosphate-buffered saline (PBS), resuspended in 6 ml of lysis solution (0.2 mg of proteinase K per ml, 0.5% sodium dodecyl sulfate [SDS], 10 mM NaCl, 1 mM EDTA, 50 mM Tris; pH 8.0), and incubated for 3 h at 37°C. Cell lysates were extracted three times with an equal volume of Tris (pH 8.0)-buffered phenol and once with chloroform-isoamyl alcohol (24:1). Chromosomal DNA was ethanol precipitated from the aqueous phase, pelleted, and resuspended in TE buffer (10 mM Tris [pH 7.6], 1 mM EDTA [pH 8.0]).

Construction and screening of an *M. gallisepticum* genomic library. Genomic *M. gallisepticum* S6 DNA was partially digested with *Sau*3AI. DNA fragments were separated on a 10 to 40% sucrose gradient. Fragments 9 to 23 kb in length were purified on a low-melting-point 1.2% agarose gel and ligated into the bacteriophage vector LambdaGEM-11 according to the manufacturer's instructions (Promega Corp., Madison, Wis.).

The resulting recombinant *M. gallisepticum* LambdaGEM-11 library was screened by using a previously described 583-bp, ^{32}P-labeled PCR fragment homologous to a portion of the P1 gene of *M. pneumoniae* and the MgPa gene of *M. genitalium* (13). Plaque lifts were hybridized at 37°C in 50% formamide for 12 h (1). Membranes were washed twice with 0.1% SDS–2× SSC (1× SSC is 0.15 M NaCl plus 0.015 M sodium citrate) for 30 min at 65°C and three times in 0.1% SDS–0.5× SSC for 30 min at 70°C. Viral DNA was purified from plaques that hybridized to the PCR probe.

Subcloning and sequencing. DNA was cloned, digested, and mapped with restriction endonucleases by standard techniques (1). Double-stranded plasmid DNA templates were sequenced with sequencing primers appropriate to Bluescript plasmids (Stratagene, La Jolla, Calif.) as well as internal oligonucleotide primers. Both strands were sequenced with Sequenase II T7 DNA polymerase (United States Biochemical Corp., Cleveland, Ohio) by the dideoxy-chain termination method (42). DNA and protein sequences were analyzed with the Sequence Analysis Software Package of the Genetics Computer Group (12).

RNA analysis. RNA was extracted from mid-log-phase cultures of *M. gallisepticum* S6 according to the procedure of Chirgwin et al. (6). Primer extension analysis was performed essentially as described by Ausubel et al. (1). Thirty picomoles of primer was end labeled with [γ-^{32}P]ATP and purified on an Elutip minicolumn (Promega Corp.). The labeled probe was hybridized to 30 μg of total RNA at 30°C for 17 h in 30 μl of hybridization buffer (1 M NaCl, 0.17 M HEPES [*N*-2-hydroxyethylpiperazine-*N*′-2-ethanesulfonic acid] [pH 7.5], 0.3 mM EDTA [pH 8.0]). After ethanol precipitation, the primer was extended with Moloney murine leukemia virus reverse transcriptase (2.5 U) for 15 min at 42°C in reverse transcriptase buffer (50 mM KCl, 5 mM $MgCl_2$, 4 mM deoxynucleoside triphosphates, 10 mM Tris [pH 8.3], 1 U of RNase inhibitor per μl). Primer extension products were phenol extracted as described above, resuspended in loading buffer, and electrophoresed for 3 h at 1,800 V on a 6% polyacrylamide sequencing gel.

Expression cloning and antibody production. A 390-bp *Hin*cII fragment of *M. gallisepticum* DNA (13) was cloned into the *Sma*I site of pGex3X, a glutathione *S*-transferase (GST) gene fusion vector (Pharmacia LKB Biotechnology, Inc., Piscataway, N.J.). Two UGA codons, which code for the amino acid tryptophan in mycoplasmas, are located near the 3′ end of the mycoplasma insert. The resulting plasmid, pGex-in, expresses a 37-kDa fusion protein (GST-MGC1) consisting of the 26-kDa GST protein fused to an 11-kDa mycoplasma polypeptide. The fusion protein was partially purified as an insoluble aggregated protein (48). A sample of the fusion protein was electrophoresed on an SDS–10% polyacrylamide gel, and the portion of the gel corresponding to the migration position of the protein was homogenized with an equal volume of Freund's complete adjuvant and injected subcutaneously and intramuscularly into New Zealand White rabbits. The rabbits were given three booster injections of the recombinant protein in Freund's incomplete adjuvant at approximately monthly intervals. Seven days after the final inoculations, the rabbits were anesthetized with ketamine hydrochloride (25 mg/kg of body weight) and xalazine (3 mg/kg) and bled by cardiac puncture. An immunoglobulin G-enriched fraction of the sera was prepared by ammonium sulfate precipitation (22), stored at −20°C, and used for immunological studies.

Trypsin sensitivity and Western blot analysis. Log-phase cultures of *M. gallisepticum* S6 were grown in Frey broth medium in 25-cm^2 plastic flasks. When the broth medium reached a pH of 7.0, the cells were washed and scraped in ice-cold PBS, pelleted at 10,000 × *g* for 10 min at 4°C, and resuspended in 8 ml of PBS. Trypsin was added to 2-ml aliquots of cells at a final concentration of 500 μg/ml, and the mixture was incubated for 5, 10, or 20 min at 37°C (32). Samples from each trypsin treatment (300 μl) were transferred to a tube containing trypsin inhibitor (type 1-S; Sigma, St. Louis, Mo.) at a concentration of 1.5 U/μg of trypsin, and the cells were pelleted by centrifugation at 10,000 × *g* for 5 min at 4°C.

The resulting cell pellets were washed once in PBS and resuspended in 200 μl of loading buffer (15% β-mercaptoethanol, 3% SDS, 0.3% bromophenol blue, 10% glycerol). Proteins (10-μl aliquots) were separated on an SDS–8 to 15% polyacrylamide gel and transferred to nitrocellulose. The membranes were blocked with BLOTTO (5% nonfat dry milk–0.03% Tween in PBS) and reacted with a 1:600 dilution of rabbit GST-MGC1 antiserum for 1 h. After two washes, the membranes were incubated in a 1:3,000 dilution of goat anti-rabbit alkaline phosphatase-conjugated antibody (Bio-Rad, Richmond, Calif.) for 30 min, washed four times with PBS, and rinsed with 100 mM Tris (pH 9.5). Bands were visualized with nitroblue tetrazolium (50 μg/ml) and 5-bromo-4-chloro-3-indolyl phosphate (100 μg/ml) in 100 mM NaCl–5 mM $MgCl_2$–100 mM Tris (pH 9.5).

Nucleotide sequence accession number. The DNA sequence of the *M. gallisepticum mgc1* gene has been determined and submitted to GenBank under accession no. U34842.

RESULTS

Identification and DNA sequence of a putative *M. gallisepticum* cytadhesin gene. A PCR-amplified 583-bp fragment of *M. gallisepticum* (13) was used to probe an *M. gallisepticum* LambdaGEM-11 genomic library. DNA from one positive clone (λ4) was purified, and Southern hybridizations (data not shown) determined that the PCR fragment hybridized to an 8.0-kb *Sac*I fragment (Fig. 1A). This *Sac*I fragment was cloned into Bluescript vector KSII, creating plasmid pMG25. Restriction mapping and additional Southern hybridizations positioned the PCR fragment within a 1.08-kb *Pst*I-*Eco*RI fragment (Fig. 1B).

Analysis of the sequence of a 3,750-bp region of pMG25, contained within five subclones, revealed a complete 3,366-nucleotide (3,366-nt) open reading frame (*mgc1*) with a G+C content of 35.3%. The initiation codon of the open reading frame is a GTG start codon (valine) 281 nt from the beginning of the sequenced region (Fig. 2A). The *mgc1* gene is flanked by open reading frames. The first 107 nt of the sequenced region encodes the carboxy-terminal 35 amino acids of a gene (ORF1) which is homologous to the *p30* gene of *M. pneumoniae*, not the ORF4 gene of the *M. pneumoniae* P1 operon (Table 1). The *mgc1* gene is separated from ORF1 by 173 nt (84.4% A+T). Another open reading frame (ORF3) begins 22 nt after the termination codon for *mgc1*.

Transcriptional analysis of *mgc1*. The transcription start site for the *mgc1* gene was determined by primer extension. An oligonucleotide primer complementary to the *mgc1* coding sequence (nt 383 to 356; Fig. 2A) was hybridized to 30 μg of total *M. gallisepticum* RNA as described in Materials and Methods. A single RNA transcript initiating at nt 76 (G) was observed (Fig. 2B). This result suggests that the 5′ end of the *mgc1* transcript initiates within the upstream open reading frame and not within the AT-rich (84.4%) 173-nt intergenic region. These results were confirmed by RNase protection assays (data not shown). No consensus promoter elements are found immediately upstream of the start of transcription, and no consensus −35 promoter sequences are found in the 173-nt region between the two open reading frames.

Comparative analysis of the MGC1 protein. The predicted translation product of *mgc1* corresponds to an unmodified protein of 1,122 amino acids with a predicted molecular mass of 121,259 Da. This protein exhibits characteristics of a class I membrane protein. The 30-amino-acid region at the amino terminus of the predicted protein (Fig. 3) contains a strongly hydrophobic core followed by a potential signal sequence cleavage site. Cleavage following Ala-30 would result in a mature protein of 118,491 Da. A potential 35-amino-acid transmembrane domain is located from residues Ala-992 to Asn-1026, near the carboxy terminus of the protein.

The *M. gallisepticum* MGC1 protein was compared with the P1 adhesin of *M. pneumoniae* and the MgPa protein of *M. genitalium*. At the nucleotide level, *mgc1* exhibits 37.4% ho-

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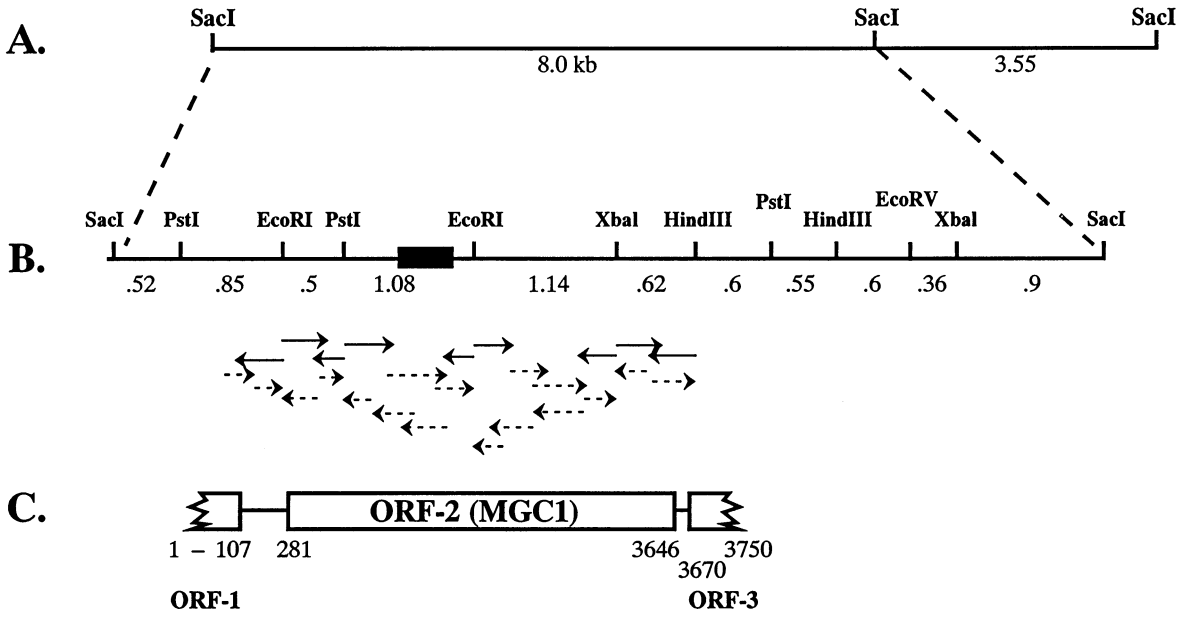

FIG. 1. Location of the *M. gallisepticum mgc1* gene. (A) The 11.55-kb region of *M. gallisepticum* cloned into LambdaGEM-11 recombinant λ4. (B) Expanded and detailed diagram of the cloned 8.0-kb *Sac*I fragment containing the *mgc1* gene (pMG25), with relevant restriction sites used in constructing subclones indicated. The location of the 583-bp PCR fragment used to identify the lambda clone (solid box) and the sequencing strategy (sequences generated by using Bluescript-specific primers [solid arrows] and sequences generated with internal primers [dashed arrows]) are shown. (C) Diagram of the gene organization of the 3,750-bp portion of pMG25 sequenced in this study, showing the relative location of *mgc1* and two flanking open reading frames.

mology to the P1 gene and 42.3% homology to the MgPa gene. At the amino acid level, MGC1 shares 28.7 and 26.3% identity to the P1 and MgPa proteins, respectively. Figure 4 graphically compares the three proteins. Although regions of homology are scattered throughout, most of the regions of extended homology are found in the carboxy-terminal portions of the proteins. Further analysis (Table 1) indicates that although MGC1 is significantly shorter than its human mycoplasma counterparts, it shares the attributes of having a proline-rich carboxy terminus, is rich in serine and threonine residues (18.2%), and lacks cysteine residues.

Expression and surface location of MGC1. The identity of the protein encoded by the open reading frame was determined by Western blotting of *M. gallisepticum* proteins. A portion of *mgc1* was cloned into the expression plasmid pGex3X, as described in Materials and Methods, creating plasmid pGex-in. Polyvalent rabbit antiserum (anti-MGC1) to the GST-MGC1 fusion protein produced in *Escherichia coli* by this plasmid was raised. Western blot analysis of log-phase cultures of *M. gallisepticum* S6 detected a single 150-kDa protein species when immunostained with this antiserum (Fig. 5, lane 1). The larger observed size of MGC1 is probably due to the proline-rich cytoplasmic domain of the protein.

As cell surface proteins, mycoplasma cytadhesins are sensitive to protease digestion. To determine if the 150-kDa MGC1 protein was surface localized, whole *M. gallisepticum* cells were treated with trypsin for various times, subjected to SDS-polyacrylamide gel electrophoresis, and immunostained with anti-MGC1. The band corresponding to MGC1 was visibly reduced with time, confirming its surface location (Fig. 5, lanes 2 to 4).

DISCUSSION

This work expands our initial study in which we identified a portion of a potential *M. gallisepticum* cytadhesin gene (13). The aims of this study were to obtain the complete nucleotide sequence of *mgc1* and to identify its protein product from *M. gallisepticum* cells.

The *M. gallisepticum mgc1* cytadhesin gene is defined by an open reading frame of 3,366 nt and 1,122 codons and starts with a valine residue. Analysis of the predicted amino acid sequence indicates that the protein has structural features in common with class I membrane proteins (47). There is a hydrophobic region of about 30 amino acids at the amino terminus of the predicted protein that has characteristics of a signal peptide (47). There is also a 35-amino-acid hydrophobic sequence near the carboxy terminus that is predicted to span the mycoplasma cell membrane. Consistent with other mycoplasma cytadhesins, MGC1 contains no cysteine residues and is rich in serine and threonine residues (Table 1). In addition, all 15 tryptophan residues in MGC1 are encoded by UGA codons.

Computer analysis reveals homology between this gene and the two major human mycoplasma cytadhesins. Most of the 26 to 29% amino acid homology is found in the carboxy-terminal region of the protein. Although epitopes involved in cytadherence are thought to be localized to the carboxy termini of these proteins, a specific 13-amino-acid *M. pneumoniae* P1 cytadherence epitope (11) is not found in MGC1. This could reflect differences in mycoplasma receptor proteins found on the surfaces of human and avian epithelial cells. Portions of the *M. pneumoniae* P1 cytadhesin gene exist as multiple or single copies (43). Only one hybridizing DNA fragment is observed when genomic *M. gallisepticum* DNA is hybridized with the internal 583-bp PCR fragment initially used to identify the gene (13), suggesting that at least part of the *M. gallisepticum mgc1* gene exists as a single copy.

The cytadhesin genes of the two human mycoplasmas are located as the second genes in three-gene operons (Table 1). The *M. gallisepticum* cytadhesin gene appears to have a different genomic structure. Northern (RNA) blot analysis suggests that *mgc1* is part of an 8.0-kb transcript (data not shown). Further examination of the sequence flanking *mgc1* suggests

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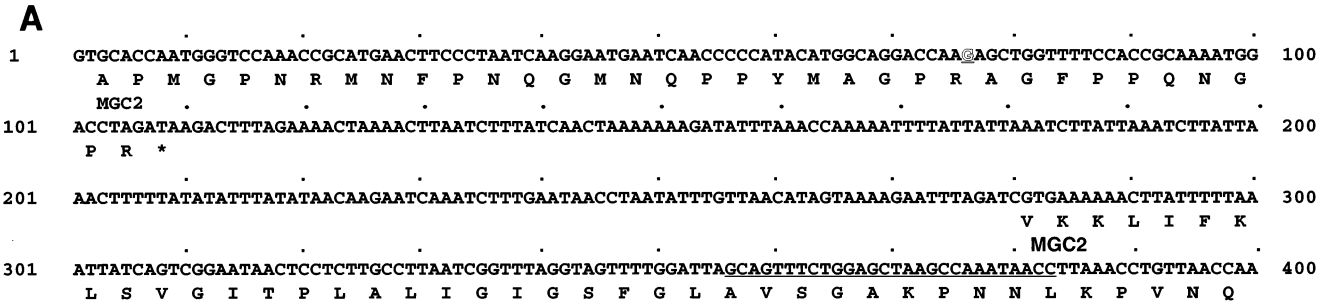

FIG. 2. (A) DNA and predicted amino acid sequences of a portion of the *M. gallisepticum mgc1* gene and a flanking open reading frame. The underlined G residue at nt 76 represents the transcription initiation site for the *mgc1* gene. The complement of the underlined sequence was used for primer extension assays. (B) Primer extension analysis of *mgc1*. The transcriptional start site for RNA synthesized in vivo was determined by the primer extension procedure using an oligonucleotide primer (within the *mgc1* coding region) as described in Materials and Methods. The DNA sequence ladder was generated by the dideoxy-chain termination method using the same primer used for the primer extension reaction. Lane P, primer extension product (arrow). The nucleotide sequence of the region is indicated on the right, with the transcription start site underlined.

that it is the first gene in at least a two-gene operon. An AT-rich, 173-nt region separates *mgc1* from an upstream open reading frame, while a 24-nt AT-rich region separates the termination codon of *mgc1* from a downstream open reading frame. A primer from within the coding region of *mgc1* was used in primer extension assays to map the 5′ end of the *mgc1* transcript. The 5′ end of the transcript maps not to the 173-nt intergenic region, as expected, but to a region within the upstream gene. These results were confirmed by RNase protection assays. The 173-nt region is 84.4% AT and has the potential to form two stem-loop structures. As for some other mycoplasma genes (45), no consensus promoter sequences were identified upstream from the start of transcription. Furthermore, the upstream open reading frame (ORF1) encodes a gene homologous to the P30 cytadhesin of *M. pneumoniae* (data not shown). This gene is not homologous to the first genes of the P1 and MgPa gene operons of *M. pneumoniae* and *M. genitalium*. Further studies of the transcriptional regulation of these two *M. gallisepticum* genes are clearly warranted.

As definitive evidence that the open reading frame encodes an authentic *M. gallisepticum* surface membrane protein, a trypsin-sensitive protein product for the gene was identified. A single 150-kDa band was detected when polyvalent rabbit an-

TABLE 1. Properties of mycoplasma adhesins[a]

Property	P1 (*M. pneumoniae*)	MgPa (*M. genitalium*)	MGC1 (*M. gallisepticum*)
Gene			
Length (nt)	4,881	4,335	3,366
Mol% G+C	53.5	39.9	35.3
Tryptophan codons (no.)	TGA (21) and TGG (16)	TGA (16) and TGG (12)	TGA (15) and TGG (0)
Genome organization	Second gene of operon (ORF4-P1-ORF6)	Second gene of operon (ORF1-MgPa-ORF3)	First gene of operon (MGC1-ORF3-?)
Protein precursor			
No. of amino acids	1,627	1,455	1,122
Mass (Da)	176,288	159,660	121,259
Leader sequence (no. of amino acids)	59	58	30
Mature protein			
Mass (Da)	169,758	153,134	118,491
Cysteine	Absent	Absent	Absent
Proline	Proline-rich C terminus	Proline-rich C terminus	Proline-rich C terminus
% serine + threonine	17.7	17.34	18.2
Trypsin sensitivity	Sensitive	Sensitive	Sensitive
Serological activity	Immunodominant	Immunodominant	Not known
Location	Clustered at tip organelle	Clustered at tip organelle	Not known

[a] Modified from the data of Razin and Jacobs (41). Data are from Dallo et al. (10, 11), Inamine et al. (27–29), Mader et al. (34), Su et al. (44), and this study.

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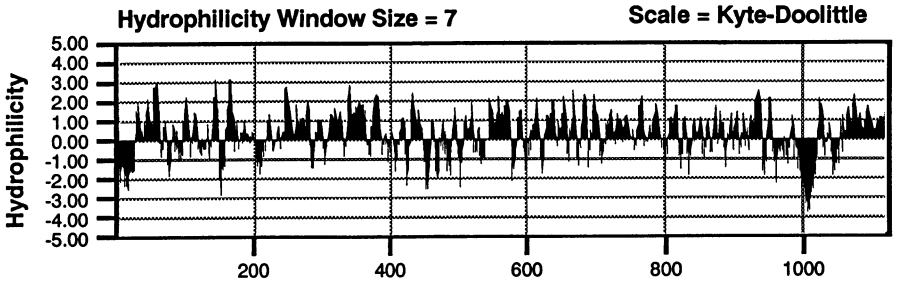

FIG. 3. Hydrophilicity plot of MGC1. Hydropathy values were calculated by the method of Kyte and Doolittle (31). Positive values indicate increasing hydrophilicity, and negative values represent increasing hydrophobicity.

tiserum against a GST-MGC1 fusion protein was used to probe Western blots of *M. gallisepticum* cell proteins. The size of this protein correlates well with the 155-kDa *M. gallisepticum* protein which Dallo and Baseman (9) found to immunologically cross-react with the cytadhesin proteins of the human mycoplasmas. Surface location was determined by treating *M. gallisepticum* cells with trypsin. The protein band corresponding to MGC1 was visibly reduced by increasing the time of trypsinization (Fig. 5, lanes 2 to 4). Similar concentrations of trypsin have been found to reduce levels of a 67-kDa *M. gallisepticum* hemagglutinin (36).

Markham et al. (21, 36, 38) have identified and characterized a hemagglutinin gene family (pMGA) from *M. gallisepticum*. The S6 strain of *M. gallisepticum* contains about 50 genes encoding pMGA-like proteins (10% of the coding capacity of the genome) (37). The 67-kDa pMGA hemagglutinin is sensitive to trypsin treatment, and monoclonal antibodies to pMGA inhibit the attachment of *M. gallisepticum* cells to avian erythrocytes. Future studies will determine if MGC1 encodes both cytadhesin and hemagglutinin activities, like the human mycoplasma cytadhesin proteins, or whether *M. gallisepticum* has distributed these functions to two separate proteins, MGC1 and pMGA.

In summary, amino acid homology and protein characteristics strongly suggest that MGC1 is an *M. gallisepticum* cytadhesin-like protein. Mycoplasmas may encode a conserved

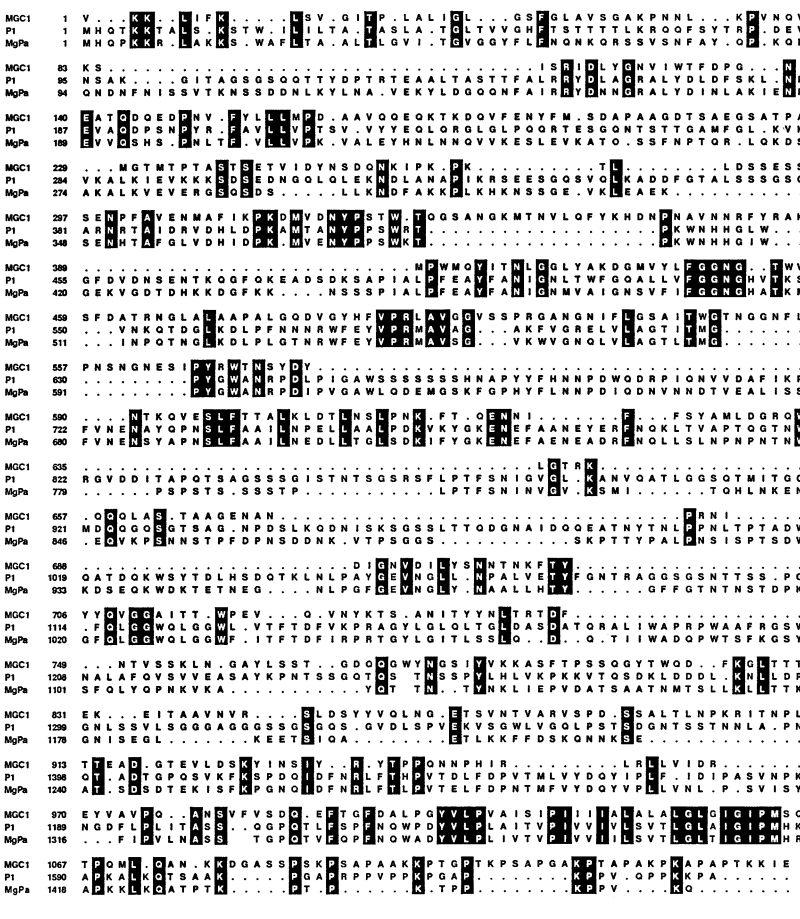

FIG. 4. Comparison of three mycoplasma adhesin proteins. Conserved residues are boxed.

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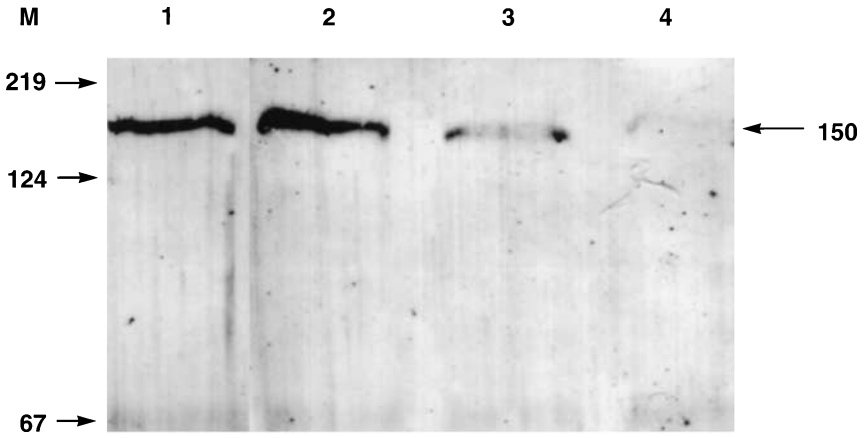

FIG. 5. Trypsin digestion of intact *M. gallisepticum*. Intact mycoplasma cells were suspended in PBS and digested with 500 μg of trypsin per ml for 0 (lane 1), 5 (lane 2), 10 (lane 3), or 20 (lane 4) min as described in Materials and Methods. Samples were electrophoresed on an SDS–8 to 15% polyacrylamide gel and immunoblotted with MGC1 antiserum. Lane M, molecular mass markers (masses shown in kilodaltons).

family of cytadhesin or cytadhesin-like proteins which have adapted to attach to different hosts. Experiments are under way to determine whether MGC1 is clustered on the tip organelle and whether MGC1 is involved in the attachment of *M. gallisepticum* to avian respiratory epithelial cells. Further studies with MGC1 and its gene may improve our understanding of mycoplasma attachment and gene regulation.

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