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Original Article

آنالیز درون رایانه‌ای پروتین pMGA1.2 در سویه‌های واکسن و بیماریزای *مایکوپلاسما گالی‌سپتیکوم*

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چکیده

سابقه و هدف: *مایکوپلاسما گالی سپتیکوم*، عامل بیماری مزمن تنفسی در جوجه‌های ماکیان، از نظر اقتصادی مهم‌ترین گونه مایکوپلاسما است که خسارات اقتصادی فراوان در سراسر جهان ایجاد می‌کند. فراوان‌ترین پروتین‌های غشایی در *مایکوپلاسما گالی‌سپتیکوم* pMGA، لیپوپروتین هایی با حدود ٦٧ کیلو دالتون می‌باشد. ژن‌های خانواده pMGA پتانسیل فوق‌العاده‌ای برای ایجاد تنوع در ساختار آنتی‌ژنی سطح سلول‌های *مایکوپلاسما گالی‌سپتیکوم* دارند. هدف از این مطالعه مقایسه الگوهای پروتین pMGA بین سویه‌ها و میزبان‌های مختلف *مایکوپلاسما گالی‌سپتیکوم* بود.

مواد و روش‌ها: ژنوم‌های کامل *مایکوپلاسما گالیسپتیکوم* در GenBank تا ژانویه ٢٠٢٠ بررسی و توالی‌های pMGA1.2 شناسایی، گروه‌بندی و کدگذاری شدند. پروتین pMGA1.2 با طول ٦٥٠ اسید آمینه بین دو میزبان مختلف (مرغ و فنچ خانگی) توسط نرم‌افزار بیوانفورماتیک در ژنوم‌های کامل *مایکوپلاسما گالی سپتیکوم* مورد بررسی قرار گرفت.

یافته‌ها: ژن pMGA1.2 در سویه‌های مختلف *مایکوپلاسما گالی‌سپتیکوم* پنج گروه اصلی با بیش از ١٠ درصد واگرایی نشان داد. بر اساس تراز چند توالی، یک الگوی خاص در جدایه فنچ خانگی شناسایی شد. جالب توجه است که دو موتیف خاص DNQNVSNQ487 ٤٨٠ و SNVSSPSY647 639 در ژن pMGA1.2 از سویه TS-11 یافت شد که می‌تواند به‌عنوان نشانگر برای شناسایی و تمایز این سویه واکسن از *مایکوپلاسما گالی سپتیکوم* بیماری‌زا استفاده شود.

نتیجه‌گیری: در این مطالعه نشان داده شد که پروتین pMGA1.2 دارای نواحی آنتی‌ژنی اپی‌توپ سلول- B است که در تمام جدایه‌ها حفظ شده است و می‌تواند در طراحی تست سرولوژیکی برای تشخیص آنتی‌بادی علیه *مایکوپلاسماگالی‌سپتیکوم* قابل استفاده باشد.

واژگان کلیدی: In silico، *مایکوپلاسما گالی سپتیکوم*، pMGA1.2.

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Original Article

In silico analysis of pMGA1.2 protein of *Mycoplasma gallisepticum* in vaccine and pathogenic strains

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Abstract

Background & Objectives: *Mycoplasma gallisepticum*, the pathogen responsible for chronic respiratory disease in chickens, is the most economically important species of *Mycoplasma that* causing tremendous economic losses worldwide. The most abundant membrane proteins in *M. gallisepticum* are pMGA, lipoproteins of about 67 kDa. The pMGA family genes have an extraordinary potential for diversifying antigenic structure on the surface of *Mycoplasma gallisepticum* cells. The aim of this study was to compare the pMGA protein patterns between different strains and hosts of *Mycoplasma gallisepticum.*

Material & Methods: All *Mycoplasma gallisepticum* full genomes available in GenBank till January 2020 were considered and $pMGA_{1.2}$ sequences were identified, grouped and coded. $pMGA_{1.2}$ protein with a chain of 650 amino acids between two different hosts (poultry and house finch) was studied by bioinformatics software in all *Mycoplasma gallisepticum* full genomes.

Results: $pMGA_{1.2}$ gene among different strains of *Mycoplasma gallisepticum* showed five major groups with more than 10 percent divergence. Based on multiple sequence alignment, a specific pattern was identified in house finch isolates. Interestingly, two specific motifs $_{480}$DNQNVSNQ$_{487}$ and $_{639}$SSNVSSPSY$_{647}$ were found in the $pMGA_{1.2}$ of TS-11 strain, which can probably be used as markers to identify and differentiate this vaccine strain from pathogenic *Mycoplasma gallisepticum*.

Conclusion: This study showed that $pMGA_{1.2}$ protein have some B-cell epitope antigenic regions that are conserved among all isolates and might be applicable to design serological test for detection antibody against *Mycoplasma gallisepticum*.

Keywords: pMGA1.2, *Mycoplasma gallisepticum*, In silico.

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Introduction

Mycoplasma gallisepticum the main respiratory pathogen of chickens and turkeys causing significant economic damages to the poultry industry (1). *Mycoplasma gallisepticum* causes respiratory rales, coughing, ocular and nasal discharge, conjunctivitis, reduction in feed intake, lower and uneven growth, decline in egg production, and increase in mortality. In addition, the

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infection may be exacerbated by complications with *E. coli* and viral pathogens, such as infectious bronchitis virus, rather than a single infection (2).

Mycoplasma gallisepticum first jumped from poultry to house finches in the early 1990s (3). This bacterial pathogen was identified as the reason of conjunctivitis in free-ranging house finches and other songbird species in the eastern United States and Canada in 1994 (4,5). By Multi Locus Sequencing Typing (MLST), identical sequence types were identified in backyard poultry and commercial poultry suggesting the potential spread of Mycoplasma gallisepticum between backyard and commercial poultry (6).

The serum agglutination reaction (SAR) test, hemagglutination inhibition (HI), and ELISA are serology tools recommended for diagnosis of *Mycoplasma gallisepticum* infection (7). Phylogenetic analyses reveal that mycoplasmas have undergone a degenerative evolution from related, low G+C content, the cell-wall-less eubacteria (8). It is well documented that *Mycoplasma gallisepticum* contains cell surface proteins with hemagglutinating (pMGA/VlhA), or adhesive properties like Mgc2, α-enolase or the uncharacterized proteins P30, P48, P50, P80 (9 , 10).

Mycoplasma gallisepticum lacks a cell wall and has only a plasma membrane, which contains about 200 proteins as the major membrane protein antigens and immunogens (11). pMGA, a surface antigen, has been explained as a lipoprotein from analysis of the gene sequence (12). Lipoproteins are targeted by the immune system (13).

Various *Mycoplasma gallisepticum* strains include 30 to 70 pMGA genes, therefore a large amount of their genome has been specified to encoding several haemagglutinins. A large part of the pMGA molecule can be bound by monoclonal antibodies on intact cells, then seems to be placed on the surface of *Mycoplasma gallisepticum* (14,15).

Past report characterized the appearance of size variants of proteins appropriated by monoclonal antibodies in *Mycoplasma gallisepticum* vaccinal F-strain (16). It has been showed that LP64 and pMGA (being probably the same protein) are involved in the attachment of *Mycoplasma gallisepticum* to the poultry respiratory epithelium (17). Three live *Mycoplasma gallisepticum* vaccines were commercially accepted including 6/85 strain, ts-11 and F-strain which have effectively reduced damages related to *Mycoplasma gallisepticum* infection in the field (18).

Attenuated vaccines stimulate immune responses by cellular and humoral immunities. TS-11 and 6/85 were commercially produced using serial passage or chemical mutagenesis while the F-strain vaccine is a naturally-attenuated field isolate. In general, F-strain derived live vaccines induce higher levels of antibodies against *M. gallisepticum* than ts-11 derived vaccines, whereas 6/85 derived live vaccines often induce low or no detectable serological response during the first months post vaccination (19).

Nine members of the pMGA gene family have been sequenced (pMGA1.1 to pMGA1.9) in *M.gallisepticum* S6 strain. High level of identity (>95%) was observed in $pMGA_{1.2}$ and pMGA1.1 genes of *Mycoplasma gallisepticum*. But other pMGA genes exhibit much lower degrees of sequence identity; however, there are districts of amino acid sequence conserved in various pMGA proteins (20).

The aim of this study was in silico analysis of $pMGA_{1.2}$ protein in order to investigate the potential of this protein in diagnosis or

Differentiate vaccinal and pathogenic strains.

Materials and methods

1. Sequence collection: Nucleotide sequence alignment was carried out using BioEdit software and MegAlign. All (18) *Mycoplasma gallisepticum* full genomes available in GenBank till January 2020 were collected at NCBI website. pMGA1.2 sequences of vaccine strains and field isolates of the bacteria were collected from GenBank databases (https://www.ncbi. nlm.nih.gov) under accession numbers CP028147 (Avipro), CP028146 (F99), CP001873 (Str.F), CP044224 (6/85), CP044225 (ts-11), CP044226 (mx-4), CP006916 (S6), LS991952 (NCTC10115), AE015450 (R_{low}) and CP001872 (R_{high}), CP003513, CP003512, CP003511, CP003510, CP003509, CP003508, CP003507 and CP003506 which were released to the public database up to Jan 1, 2020.

2. Multiple Alignments, sequences analysis and Phylogenetic Analysis: Multiple sequence alignments using ClustalW method of MegAlign software were performed in order to identify $pMGA_{1.2}$ sequences. The FASTA and BLAST programs (BLASTN and BLASTP) were used to search the DNA and protein databases. Nucleotide identity of the pMGA1.2 sequences of *Mycoplasma gallisepticum* full genomes was determined using the nucleotide BLAST algorithm with GenBank database (http://www.ncbi.nlm.nih.gov). Nucleotide and protein sequences of $pMGA_{1.2}$ were compared and the percentage of divergence was calculated by MegAlign software. Deletion, insertion, amino acid substitutions in different strains and host groups were evaluated. The alignment of nucleotide sequences of $pMGA_{1.2}$ genes were detected based on the phylogenetic tree.

3. Epitopes Prediction: Antigenic regions and Linear B-cells epitopes were predicted in different strains of $pMGA_{1.2}$ protein by online IEDB (Immune Epitope Database and Analysis Resource) software http://www.iedb.org/.

Results

1. Multiple Alignment of $pMGA_{1.2}$ Protein in different strains: Sequences of $pMGA_{1.2}$ protein were analyzed by MegAlign softwares. The phylogenetic tree of the $pMGA_{1.2}$ gene among different strains of *Mycoplasma gallisepticum* showed five major groups with more than 10 percent divergence: group A: include house finch isolates, B: include F strains (vaccine strains), C: 6.85 strain, D: S6 and R strains, and E: TS-11 strains (Vaccine strains) (Fig.1, Table1).

Ten insertion/deletion in amino acid level, 39 single amino acid variation (SAV) and 50 single amino acid polymorphism (SAP) were observed based on multiple alignment of $pMGA_{1.2}$ protein among different strains.%100 similarity among house finch isolates was observed. The minimum similarity is related to strain S6 and TS-11 strain. The divergence between these two strains is more than 10%.

A repeated $_{29}$PTPNPTPN$_{36}$ sequence was observed in position 28 to 36 of the $PMGA_{1.2}$ protein. The sequences are divided into two major groups based on this repeated motif. The sequences of house finch and F and S6 strains were repeated twice and the rest showed one repetition. In the TS-11 strain, one repeat of a "$_{29}$PTPNPTPN$_{36}$" sequence, a T_{28} amino acid deletion and an N_{42} insertion were observed. Two specific amino acids I_{76} and N_{95} were observed in two strains MX-4 and S6.

Two specific motifs $_{480}$DNQNVSNQ$_{487}$ and

$_{639}$SSNVSSPSY$_{647}$ were found only in the sequence of TS-11 strain, which can probably
be used as markers to identify this strain. In addition, two other specific amino acid markers, $_{499}$SS$_{500}$ and S$_{507}$ were observed in this strain.

A specific pattern was also observed in F strain. Specific amino acids T$_{374}$, N$_{555}$, A$_{560}$, S$_{568}$, E$_{572}$, A$_{593}$, Q$_{596}$, $_{600}$VAN$_{602}$ and D$_{648}$ were identified in F strains which can be used as markers to identify F strains. Other specific pattern with eleven amino acids S$_{184}$, K$_{453}$, N$_{478}$, K$_{510}$, M$_{513}$, I$_{531}$, R$_{551}$, H$_{562}$, L$_{610}$, S$_{630}$, and S$_{641}$ identified in house finch isolates.

Comparison of pMGA$_{1.2}$ protein sequence of strain S6 with other sequences showed seven specific amino acids K$_3$, S$_{295}$, V$_{358}$, K$_{371}$, D$_{380}$, R$_{538}$, E$_{539}$ and G$_{553}$ for S6. Three specific amino acids K$_{46}$, G$_{78}$ and I$_{399}$ were found in MX-4 strain.

2. Antigenic region and Epitope prediction: Based on IEDB immunoinformatics, databases 21 to 24 epitopes were identified on pMGA$_{1.2}$ protein (Threshold 1.007, Kolaskar & Tongaonkar method) (table2). Two specific linear B-cell epitopes for MX-4 and S6 strains and four specific epitopes for Fstrains were observed. The results showed that despite the amino acid differences between the strains, there are the same antigenic parts between the all strains, and this evidence has a good potential in using the structure of pMGA protein in serological tests to identify antibodies against Mycoplasma.

Bioinformatics studies show that this protein is stable (instability index 27) and has resistance to temperature as well as good solubility (hydropathicity -0.408).

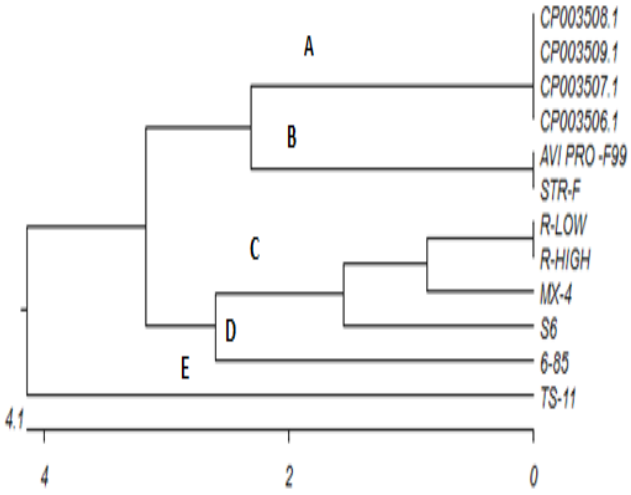


Fig 1: A phylogenetic tree based on the alignment of the protein sequences of the pMGA$_{1.2}$ gene in strains of *Mycoplasma gallisepticum.*

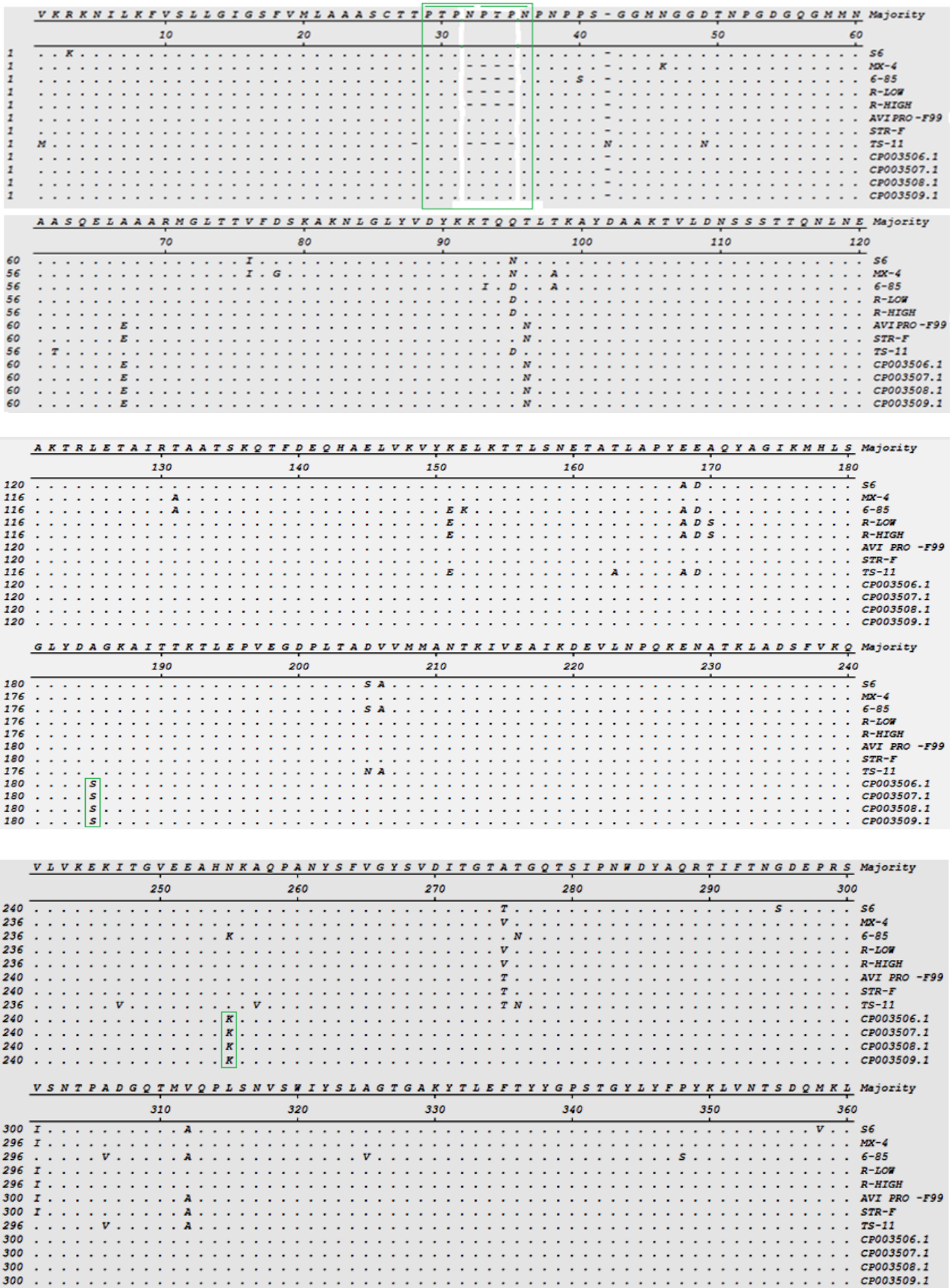
Table 1: Percent of identity and divergence in pMGA protein between different strains.

Percent Identity

	1	2	3	4	5	6	7	8	9	10	11	12		
1		96.7	94.6	97.2	97.2	92.4	92.4	88.5	92.9	92.9	92.9	92.9	1	S6
2	3.3		95.0	98.3	98.3	93.3	93.3	88.7	93.5	93.5	93.5	93.5	2	MX-4
3	5.7	5.1		95.8	95.8	93.6	93.6	91.5	93.5	93.5	93.5	93.5	3	6-85
4	2.8	1.7	4.3		100.0	93.6	93.6	90.1	93.8	93.8	93.8	93.8	4	R-LOW
5	2.8	1.7	4.3	0.0		93.6	93.6	90.1	93.8	93.8	93.8	93.8	5	R-HIGH
6	6.5	5.5	6.3	5.1	5.1		100.0	92.0	95.2	95.2	95.2	95.2	6	AVI PRO -F99
7	6.5	5.5	6.3	5.1	5.1	0.0		92.0	95.2	95.2	95.2	95.2	7	STR-F
8	9.5	10.0	7.5	8.4	8.4	7.9	7.9		91.3	91.3	91.3	91.3	8	TS-11
9	7.1	6.3	6.5	6.0	6.0	4.6	4.6	8.4		100.0	100.0	100.0	9	CP003506.1
10	7.1	6.3	6.5	6.0	6.0	4.6	4.6	8.4	0.0		100.0	100.0	10	CP003507.1
11	7.1	6.3	6.5	6.0	6.0	4.6	4.6	8.4	0.0	0.0		100.0	11	CP003508.1
12	7.1	6.3	6.5	6.0	6.0	4.6	4.6	8.4	0.0	0.0	0.0		12	CP003509.1
	1	2	3	4	5	6	7	8	9	10	11	12		

Divergence

A higher similarity in the pMGA protein structure is observed between F vaccine and pathogenic strains s6, MX-4 compared to ts-11 vaccine strain (table 1).


Majority
S6
MX-4
6-85
R-LOW
R-HIGH
AVI PRO -F99
STR-F
TS-11
CP003506.1
CP003507.1
CP003508.1
CP003509.1

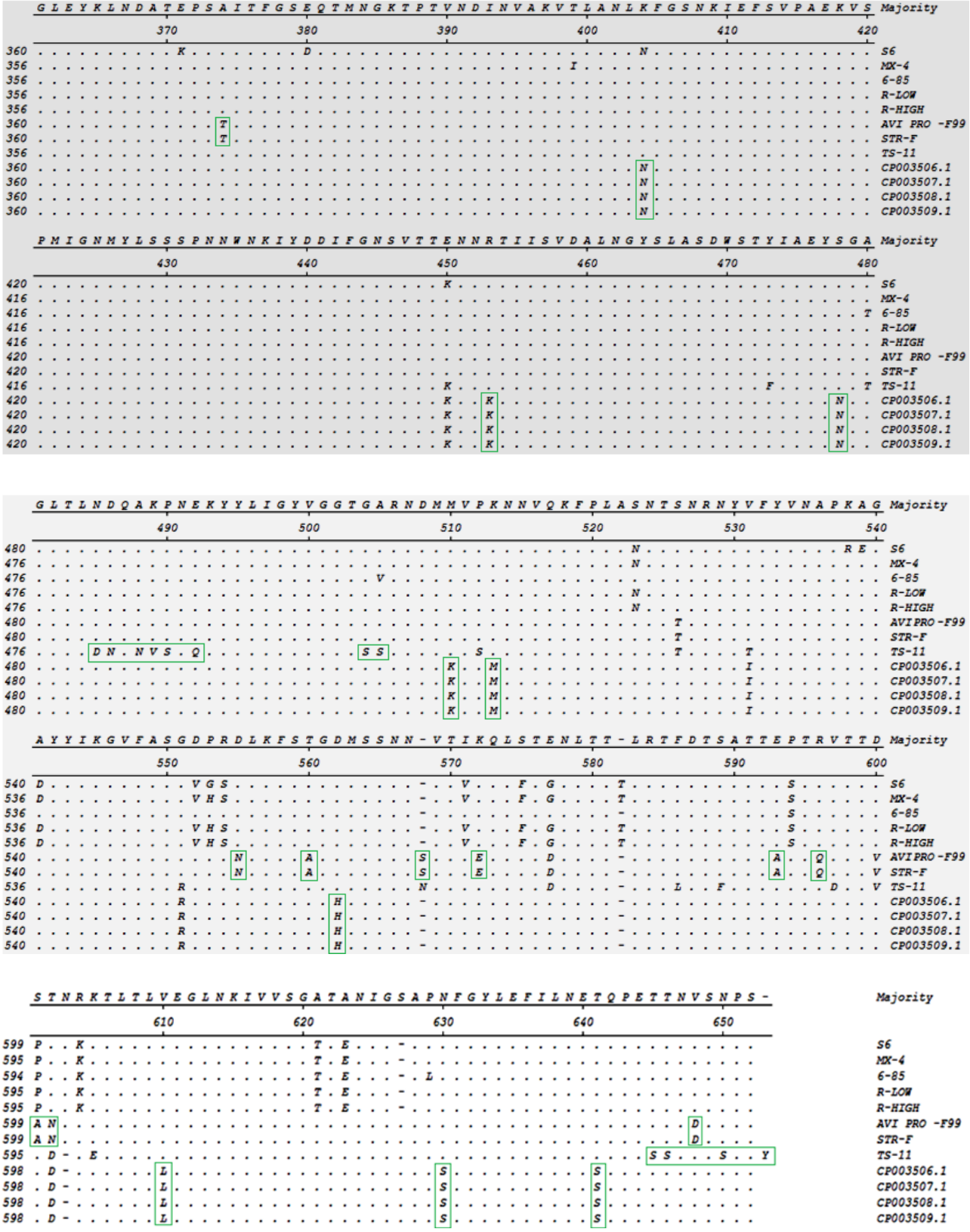


Fig 2: Alignment of the amino acid sequences of the pMGA$_{1.2}$ gene derived from corresponding nucleotide coding sequences (GenBank accession number L28424).

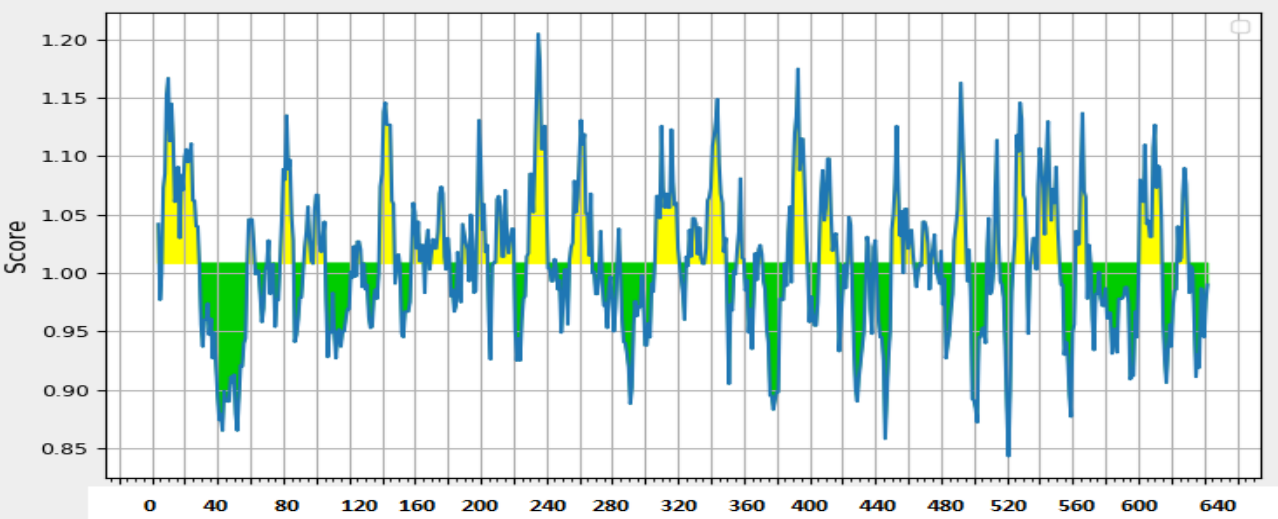


Fig 3: Antigenic regions of $pMGA_{1.2}$ protein.

Table 2: Linear B-cell Epitope predicted in different strains of $pMGA_{1.2}$

Predicted peptides: mx-4

Start	End	Peptide
7	28	LKFVSLLGIGSFVMLAAASCTT
78	86	NLGLYVDYK
99	105	AKTVLDN
139	147	AELVKVYKE
158	165	TLAPYEEA
170	178	IKMHLSGLY
198	204	TADVVMM
208	220	KIVEAIKDEVLNP
228	240	LADSFVKQVLVKE
254	267	PANYSFVGYSVDIT
306	320	MVQPLSNVSWIYSLA
327	334	TLEFTYYG
337	349	TGYLYFPYKLVNT
390	398	VAKVILANL
406	417	EFSVPAEKVSPM
450	456	IISVDAL
458	463	GYSLAS
488	495	KYYLIGYV
524	532	NYVFYVNAP
539	552	IKGVFASGVHSDLK
562	568	NVTVKQL
601	614	LTLVEGLNKIVVSG
624	630	FGYLEFI

Predicted peptides:S6

Start	End	Peptide
6	28	ILKFVSLLGIGSFVMLAAASCTT
82	90	NLGLYVDYK
98	100	KAYDAAKTVLDN
143	151	AELVKVYKE
162	172	TLAPYADAQYA
174	182	IKMHLSGLY
212	224	KIVEAIKDEVLNP
232	244	LADSFVKQVLVKE
258	270	PANYSFVGYSVDI
314	325	LSNVSWIYSLAG
331	354	TLEFTYYGPSTGYLYFPYKLVNTS
356	364	QVKLGLEYK
394	402	VAKVTLANL
410	421	EFSVPAEKVSPM
454	460	IISVDAL
462	467	GYSLAS
492	499	KYYLIGYV
528	535	NYVFYVNA
543	554	IKGVFASGVGSD
566	572	NVTVKQL
605	618	LTLVEGLNKIVVSG
628	634	FGYLEFI

Predicted peptides:Str F

Start	End	Peptide
7	29	LKFVSLLGIGSFVMLAAASCTTP
73	78	TTVFDS
82	90	NLGLYVDYK
99	109	AYDAAKTVLDN
143	151	AELVKVYKE
162	169	TLAPYEEA
174	182	IKMHLSGLY
202	208	TADVVMM
211	224	TKIVEAIKDEVLNP
232	244	LADSFVKQVLVKE
258	270	PANYSFVGYSVDI
314	325	LSNVSWIYSLAG
329	353	KYTLEFTYYGPSTGYLYFPYKLVNT
394	402	VAKVTLANL
410	421	EFSVPAEKVSPM
454	460	IISVDAL
462	467	GYSLAS
471	476	TYIAEY
492	499	KYYLIGYV
529	536	YVFYVNAP
538	540	AGAYYIKGVFAS
592	598	PTQVTTV
605	619	LTLVEGLNKIVVSGA
628	635	NFGYLEFI

Predicted peptides:TS-11

Start	End	Peptide
7	29	LKFVSLLGIGSFVMLAAASCTPT
69	74	TTVFDS
78	86	NLGLYVDYK
93	105	TKAYDAAKTVLDN
139	147	AELVKVYEE
158	168	ALAPYADAQYA
170	178	IKMHLSGLY
208	220	KIVEAIKDEVLNP
228	245	LADSFVKQVLVKEKVTGV
254	266	PANYSFVGYSVDI
310	321	LSNVSWIYSLAG
327	349	TLEFTYYGPSTGYLYFPYKLVNT
390	398	VAKVTLANL
406	417	EFSVPAEKVSPM
450	456	IISVDAL
458	463	GYSLAS
486	497	NQKYYLIGYVGG
526	532	IFYVNAP
534	545	AGAYYIKGVFAS
600	614	LTLVEGLNKIVVSGA
624	630	FGYLEFI

Predicted peptides:HOUSE FINCH

Start	End	Peptide
7	28	LKFVSLLGIGSFVMLAAASCTT
73	78	TTVFDS
82	90	NLGLYVDYK
99	109	AYDAAKTVLDN
143	151	AELVKVYKE
162	169	TLAPYEEA
174	181	IKMHLSGL
202	208	TADVVMM
212	224	KIVEAIKDEVLNP
232	244	LADSFVKQVLVKE
258	271	PANYSFVGYSVDIT
310	324	MVQPLSNVSWIYSLA
331	353	TLEFTYYGPSTGYLYFPYKLVNT
394	402	VAKVTLANL
410	421	EFSVPAEKVSPM
454	460	IISVDAL
462	467	GYSLAS
492	499	KYYLIGYV
517	522	KFPLAS
530	536	IFYVNAP
538	540	AGAYYIKGVFAS
603	617	LTLEGLNKIVVSGA
626	633	SFGYLEFI

Discussion

Mycoplasma gallisepticum is considered one of the most costly avian diseases to the poultry industry all over the world which is mostly due to egg generation losses (21).

Mycoplasma gallisepticum infections are classified as sporadic by the USDA Animal Plant and Health Inspection Service (2013). Vaccination is propounded the most practicable way of controlling diseases, where depopulation of animals is impracticable, particularly on wide commercial multi-age

layer facilities (22).

A multi-gene family in mycoplasmas is that encoding the pMGA cell-surface-exposed lipoproteins. Contrary to the presence of many pMGA genes, all but one either is transcriptionally silent or is transcribed at very low levels within individual field isolates of the organism (23).

Based on previous reports the intergenic regions 5` to the 9.2 and 9.3 ORF contain 15 and 20 copies of the GAA trinucleotide repeats which are characteristic of the intergenic areas of pMGA family members. Variation in the number of GAA repeats is a mechanism of transcriptional regulation for the pMGA genes and 12 GAA repeats are present in the intergenic regions of those pMGA genes that are expressed (24).

In the comparison of pMGA gene expression in different *Mycoplasma gallisepticum* strains received from infected chickens, Berlic et al. (2000) proposed that two kinds of $pMGA_{1.1}$/ $pMGA_{1.2}$ genes could be distinguished in various strains relying the presence or absence of a repeat sequence in the N-terminal proline-rich area. Prolin rich regions of proteins can make elongated structures that may action in protein-protein interactions (25).

In our study, a difference of 18 nucleotides and 6 amino acids length was observed in the $pMGA_{1.2}$ gene and protein respectively. The presence of repeated sequence $_{29}PTPNPTPN_{36}$ in the F vaccine strain and the absence of the $_{29}PTPNPTPN_{36}$ repeated sequence in the TS-11, R-High vaccine strains and pathogenic R-Low indicate the lack of correlation between this sequence and bacterial virulence. All the House-finch isolates had two repetitions of the $_{29}PTPNPTPN_{36}$ sequence, which indicates that they are all from the same lineage.

In *Mycoplasma gallisepticum*, the pMGA gene sequences have been reported only for strains S6 and PG31 (26). Moreover, the N-terminal amino acid sequencing of the pMGA has been done for the F and R strains and confirmed a synthesis of proteins similar to the $pMGA_{1.2}$ .Three different strains of *Mycoplasma gallisepticum*, S6, R, and F, expressed single, unique variants of pMGA (27).

Yasmin et al in 2018 indicated the presence of pMGA and pvpA gene sequences and size variations among MG field strains from Malaysia. Their study was on the basis of pMGA and pvpA partial nucleotide sequence.

Patterns of pMGA expression and occurrence of antigenically distinct $pMGA_{1.9}$ protein have been previously investigated only in a highly passaged S6 strain (28).

With the worldwide increase in live vaccine usage to control *Mycoplasma gallisepticum*, techniques that allow differentiation of wild-type (field strain) *Mycoplasma gallisepticum* from the vaccine strains are important. In addition, strain differentiation techniques maybe used as valuable tools for the control of *Mycoplasma gallisepticum,* facilitating rapid recognition of outbreaks and epidemiological traceability (29).

In our study, two motifs $_{480}DNQNVSNQ_{487}$ and $_{639}SSNVSSPSY_{647}$ were found specific for TS-11 vaccine strain. It seems that, the nucleotide sequences of these two markers (1461-1440) and (1941-1887) can be used to design a specific primer to differentiate the TS-11 vaccine strain from other based on PCR technique. In this case, a product of about 480 base pairs will represent the vaccine strain.

More study is needed to evaluate the performance of these two TS-11 specific motifs in DIVA test for differentiation of infected from vaccinated chicken with TS-11.

Live vaccines often showed pathogenicity and adverse side effects, while bacterins have high cost and often repeated doses are required to boost avian immune system. Hence, new novel recombinant vaccines are needed to be developed which are more efficacious and less expensive. More complex vaccines consist of one or more purified antigens, killed pathogens or bacterins with an adjuvant that stimulate the immune response. These vaccines are protective but their utilization is limited due to cost (30).

In silico analysis of vaccine candidate antigens is helpful for design of new generation vaccines. Bioinformatics tools for predicting antigenic properties and candidate vaccines analysis are now a standard approach. Several bioinformatics software and servers are available that can help in the process for planning of chimeric vaccine design (31,32).

Research on in silico approaches had not yet been reported for *Mycoplasma gallisepticum* while, some researchers used in silico approaches for the identification of virulence candidates for other *mycoplasma* species such as *Mycoplasma pneumoniae* type 2a strain 309 and *Mycoplasma agalactiae* (33,34). Very few studies are reported in the field of in silico vaccine for poultry and animals. The following studies validate the immunoinformatics method to design multi-epitopic vaccines against infectious diseases in poultry. The in silico validations like molecular docking and in silico cloning and immune simulation were used in indian study to make the constructed multi-epitope as functionally suitable as a vaccine candidate against *M. gallisepticum* infection(35). In other study, researchers have reported the primary, secondary, and tertiary structural characteristics and subcellular localization, presence of the transmembrane helix and signal peptide, and functional characteristics of vlhA proteins from *M. gallisepticum* strain R low (36). Thus, future studies are recommended on in silico approaches for the development of effective vaccines. In our study, was shown that $pMGA_{1.2}$ protein have some B-cell epitope antigenic regions that are conserved among all isolates and might be applicable to design a universal immunogenic antigen as a recombinant vaccine or applicable to designing serological test for detection antibody against *Mycoplasma gallisepticum*.

Conclusion

$pMGA_{1.2}$ gene showed five groups with more than 10 percent divergence among different strains of *Mycoplasma gallisepticum*. In this study was shown that $pMGA_{1.2}$ protein have some B-cell epitope antigenic regions that are conserved among all isolates and might be applicable to design serological test for detection antibody against *Mycoplasma gallisepticum*.

Interestingly, on the other hand, two specific amino acid sequences $_{480}$DNQNVSNQ$_{487}$ and $_{639}$SSNVSSPSY$_{647}$ were found in the $pMGA_{1.2}$ protein of TS-11 strain, which can might be used as markers to identify this vaccine strain and differentiate from other.

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Conflict of interest

The authors declare no conflicts of interest.

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