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Research Article

Molecular characterization and T and B cell epitopes prediction of *Mycoplasma synoviae* 53 strain VlhA hemagglutinin

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Abstract

Mycoplasma sinoviae is a major pathogen of poultry causing synovitis and respiratory infection. *M. synoviae* hemagglutinin (VlhA) is a lipoprotein encoded by related multigene families that appear to have arisen by horizontal gene transfer. It is an abundant immunodominant surface protein involved in host-parasite interaction mediating binding to host erythrocytes. Herein, we have performed *in silico* analysis of the *vlh*A gene product from the *Mycoplasma synoviae* 53 strain and compared it to the VlhA protein of *M. synoviae* WUV1853 strain. The VlhA of the *M. synoviae* 53 strain possesses 569 amino acids and showed 85% identity with the VlhA protein of the *M. synoviae* WUV1853 strain. Further, a signal peptide was identified from amino acid M_1 to D_{28} and a cleavage site between D_{28} and Q_{29}, both located in the N-terminal domain of the molecule. Additionally, an insertion of PAPT amino acids was observed between T_{30}-P_{35} and a deletion of the amino acids GTPGNP within the PRR region of the VlhA from the *M. synoviae* 53 strain, which may be related to its reduced virulence. Finally, we have identified 17 B cell epitopes and 22 T cells epitopes within the VlhA from the *M. synoviae* 53 strain. The B cell epitope S_{263}-D_{277} and the T cell epitopes N_{45}-N_{54} and G_{58}-N_{67} showed 100% and 87-100% identity, respectively, with regions of VlhA protein of tested *Mycoplasma synoviae* and *Mycoplasma galisepticum* strains. Thus, these peptides represent new candidate molecules for the development of efficient diagnostic assays and new subunit vaccines.

Key words: Mycoplasma synoviae, hemagglutinin, epitopes, host-parasite interaction, vaccine.

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Introduction

Mycoplasma synoviae is one of the smallest and simplest bacteria lacking a cell wall known to exist, and it is a major pathogen of chickens and turkeys, causing respiratory tract infection and arthritis worldwide (Kleven, 1997). Although the basis of mycoplasma pathogenicity remains unknown, it is widely accepted that most of the damage resulting from mycoplasma infections in humans and animals is due to host immune and inflammatory responses rather than to direct toxic effects of mycoplasma virulence factors (Razin *et al*., 1998).

M. synoviae isolates differ in their infectivity, tissue tropism and pathogenicity (Rottem, 2003). Many animal mycoplasmas depend on adhesion to host tissues for colonization and infection. In these mycoplasmas, adherence is the major virulence factor and adherence-deficient mutants are avirulent (Baseman and Trully, 1997). Current theory holds that mycoplasma remains attached to the surface of epithelial cells, although some mycoplasmas have evolved mechanisms for entering host cells that are not naturally phagocytic (Razin *et al.*, 1998). The intracellular localization is obviously a privileged niche, well protected from humoral mechanisms of the host immune system and from the action of many antibiotics. The finding that some mycoplasmas can reside intracellularly opens up new horizons to the study of the role of mycoplasma and host surface molecules in invasion. Although, the ability of internalized mycoplasmas to multiply within the host cell remains to be convincingly demonstrated, reports describing mycoplasma invasive phenotypes have offered new insights into the potential virulence strategies employed by these bacteria (Rottem, 2003).

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Escaping the host immune system is of critical importance to mycoplasma survival within its host. The major survival mechanisms that have been extensively studied are molecular mimicry and phenotype plasticity which ensure that mycoplasmas are not fully nor efficiently recognized by the host immune system (Markham *et al.*, 1994; Wren, 2000). Molecular mimicry refers to antigenic epitopes that are shared by different mycoplasmas and host cells and they are considered as putative factors involved in the evasion of host defense mechanisms (Rottem, 2003). Mycoplasmas are also endowed with phenotypic plasticity defined as the ability of a single genotype to change its antigenic make-up to produce more than one morphology, physiological state, and/or behavior in response to environmental conditions (Rottem, 2003). The common way to achieve phenotype plasticity in mycoplasma is by antigenic variation. Additionally, membrane lipoproteins are the major components of intact mycoplasmas and are able to activate macrophages, thus playing an important role in cytokine production and consequently in the inflammatory response during infection (Chambaud *et al.*, 1999).

VlhA is a variable protein encoded by the *vlhA* gene in *M. synoviae* that is post-translationally cleaved into the N-terminal lipoprotein fragment MSPB (Major Surface Protein B) and the C-terminal fragment MSPA (Major Surface Protein A) which is directly involved in hemadherence (Noormohammadi *et al.*, 1997). There is only one copy of the complete *vlhA* gene in the genome of the *M. synoviae* WUV1853 strain. Other copies are not functional genes and lack the 5' end of the expressed gene (Noormohammadi *et al*., 1997, 2000). Comparing different *M. synoviae* strains, it was possible to observe differences in length and antigenic determinants of MSPB proteins (Noormohammadi *et al*., 1997). The complete genome sequence of *Mycoplasma synoviae* 53 strain revealed the organization of hemagglutinin genes with a single locus comprising 70 coding DNA sequences (CDS) (Vasconcelos *et al*., 2005). In this study, we have characterized the *vlh*A gene product from the *Mycoplasma synoviae* 53 strain and compared it to the VlhA protein of the *Mycoplasma synoviae* WUV1853 strain (Noormohammadi *et al*., 1997). The VlhA of the *Mycoplasma synoviae* 53 strain possesses 569 amino acids, a signal peptide and a cleavage site located in the N-terminal domain of the molecule. Additionally, using bioinformatic search tools, we have identified 17 B cell epitopes and 22 T cells epitopes that may be involved in host immune response against this microorganism.

Materials and Methods

DNA and amino acid sequences

The DNA and translated amino acid sequences of *vlhA* genes from *Mycoplasma synoviae* 53 (Vasconcelos *et al.*, 2005) and *Mycoplasma synoviae* WUV1853 strains (Noormohammadi *et al.*, 1997) were retrieved from GenBank under accession no. NC007294 and AF035624, respectively. The *vlhA* gene is located between the nucleotides of number 292135 and 293844 of the genome sequence of the *Mycoplasma synoviae* 53 strain sequenced by our group (Vasconcelos *et al.*, 2005).

Characterization of *M. synoviae* VlhA by bioinformatics

These amino acid sequences for VlhA from *Mycoplasma synoviae* 53 and *Mycoplasma synoviae* WUV1853 strains were aligned by CLUSTALW Multiple Sequence Alignment available online. SOSUISignal and SOSUI were used to identify motifs in these proteins, such as peptide signal and hydrophobic domains. Additionally, SignalP 3.0 software was used for prediction of cleavage sites.

T and B cell epitopes prediction

The B cell epitope prediction was performed using the program Predicting Antigenic Peptides available online. The software for the detection of antigenic peptides is based on Kolaskar's and Tongaonkar's method previously described (Kolaskar and Tongaonkar, 1990). The T cell epitope prediction was performed using RANKPEP software. This software uses Position Specific Scoring Matrices (PSSMs) or profiles from a set of aligned peptides known to bind to a given MHC molecule as the predictor of MCH-peptide binding. We used the mouse MHC system H-2, as a model for this study and tested the I-A^b and I-A^k alleles for MHC class II. Herein, we have selected several peptides that had high scores of binding to these MHC class II alleles. Predicted T and B cell epitopes shared between *M. synoviae* 53 and *M. synoviae* WUV1853 strains were also analyzed for their identity with other *Mycoplasma synoviae* strains (*Mycoplasma synoviae* B133-96, B154-02, B2700, B31-88, B38-96-170, B94-91, J26-85, J151-85, K1, K4, K1968, K2581, K27, MS-H, TN/427, ULB925 and ULB925KF) and *Mycoplasma gallisepticum* strains (*M. gallisepticum* R and S6) using the BLAST computer program blastp.

Results and Discussion

Mycoplasma synoviae is a major pathogen of chickens and turkeys that causes great economic losses in intensive poultry production. This bacterium synthesizes hemagglutinin VhlA, an abundant immunodominant surface lipoprotein. In most *M. synoviae* strains, the hemagglutinin VlhA is cleaved into N-terminal region (MSPB) and a C-terminal region (MSPA), which mediates erythrocytes binding (Bencina *et al.*, 2001). MSPB has been divided into two domains: a conserved and a variable region (Bencina *et al.*, 2001). In different *M. synoviae* isolates, MSPB proteins differ in length as well as antigenic determinants defined by monoclonal antibodies and also differ in the insertion or deletion of amino acid sequences within the proline-rich re-

peats (PRR) region which has been identified in immunodominant surface antigens involved in the interaction between pathogens and host cells (Noormohammadi *et al.*, 1997; Bencina *et al.*, 2001). A longer PRR region has been associated with higher invasiveness for the *M. synoviae* strain K1968 (Bencina *et al*., 2001). Further, *M. synoviae* clonal populations can synthesize size and antigenic variants of MSPB proteins and their expression can be associated with transition from HA^+ to HA^- phenotype (Noormohammadi *et al.*,1997).

The 569 amino acid sequence of VlhA from the *M. synoviae* 53 strain and the 785 amino acid sequence of the VlhA protein derived from the *M. synoviae* WUV1853 strain were retrieved from the GenBank and aligned in order to compare their identity. VlhA protein of *M. synoviae* 53 strain showed 85% identity with VlhA of the *M. synoviae* WUV1853 strain, despite the difference in length (Figure 1). Amino acid sequence analysis performed by SOSUISignal and SignalP 3.0 computer programs resulted in the identification of a region encoding a signal peptide from amino acid M_1 to D_{28} and a cleavage site between D_{28} and Q_{29} in the VlhA of the *M. synoviae* 53 strain that are also present and they correspond to the same positions in the VlhA of the *M. synoviae* WUV1853 strain. No variation in the signal peptide domains of both proteins was detected. The cleavage site which results in the MSPB and MSPA membrane antigens is located at S_{306} in the VlhA of the *M. synoviae* 53 strain and at S_{308} in the VlhA of the *M. synoviae* WUV1853 strain (Figure 2). Among the differences between the two sequences, there is an insertion of four amino acids (PAPT) after T_{30} of VhlA from the *M. synoviae* 53 strain This insertion is similar to an insertion observed in the *M. synoviae* FMT strain which is considered mildly pathogenic (Bencina *et al*., 2001). The MSPB domain of the *M. synoviae* 53 strain has also a deletion of six amino acids within the proline-rich repeats (PRR) (GTPGNP) in the N-terminal region which correlates to amino acids G_{54} to P_{59} of the *M. synoviae* WUV1853 strain (Figures 1 and 2). This deletion may affect bacterial virulence. The live attenuated *M. synoviae* MS-H strain vaccine has a similar deletion of the amino acids PGNPGT within the PRR region

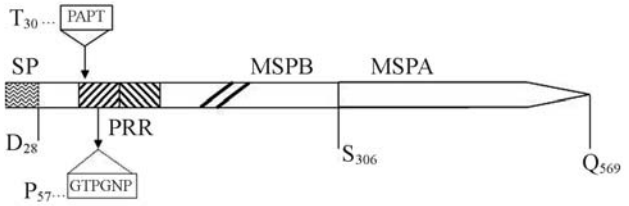

Figure 2 - Schematic representation of VlhA protein of *M. synoviae* 53 strain. Signal peptide region (SP) comprises amino acids from M_1 to D_{28}. The MSPB region contains two proline-rich repeats region (PRR) and has a PAPT insertion at T_{30} and a GTPGNP deletion at P_{57}. D_{28} and S_{306} indicate signal peptide and VlhA cleavage site, respectively.

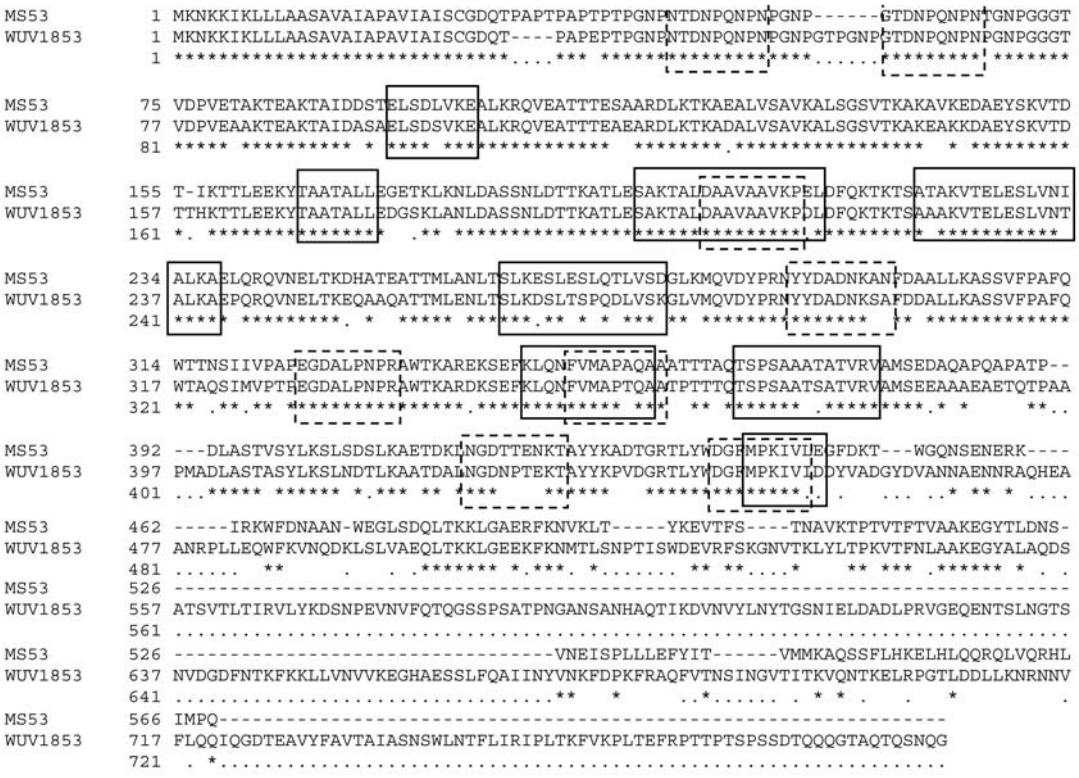

Figure 1 - Comparison of the deduced amino acid sequences of VlhA of *Mycoplasma synoviae* strain 53 (GenBank accession number NC007294) and *Mycoplasma synoviae* strain WUV1853 (GenBank accession number AF035624). Asterisks indicate identical residues and gaps are indicated by dashes. Squares with dashed-lines represent T cell epitopes and squares with solid-lines represent B cell epitopes.

Table 1 - B cell epitopes predicted in the VlhA protein of *M. synoviae* 53 strain.

Sequence	Position
ELSDLVKE	94-101
AEALVSAVKALSGSVTKAKAVKE	123-145
EYSKVTD	148-154
TAATALL	165-171
SAKTALDAAVAAVKPEL	195-211*
ATAKVTELESLVNIALKA	220-237
SLKESLESLQTLVSD	263-277
LKMQVDYPR	279-287
NSIIVPA	317-323
KLQNFVMAPAQA	345-356*
TSPSAAATATVRV	364-376
QAPQAPATPDLASTVSYLKSLSD	383-405
MPKIVLE	440-446*
KNVKLTYKEV	489-498
AVKTPTVTFTVAA	504-516
NEISPLLLEFYITV	527-540
QSSFLHKELHLQQRQLVQRHL	545-565

*Protein regions containing B and T cell epitopes (Figure 1).

Table 2 - T cell epitopes predicted in the VlhA protein of *M. synoviae* 53 strain.

Sequence	Position	Score[a]	Haplotype
PAPTPAPTP	31-39	12.8	I-A^b
APTPTPGNP	36-44	13.5	I-A^b
NTDNPQNPN	45-53	20.7	I-A^k
DNPQNPNPG	47-55	9.36	I-A^k
GTDNPQNPN	58-66	18.2	I-A^k
NTGNPGGGT	66-74	9.63	I-A^k
KTALDAAVA	197-205	12.69	I-A^b
DAAVAAVKP	201-209	12.6	I-A^b
YYDADNKAN	289-297	13.9	I-A^b
DADNKANFD	291-299	14.2	I-A^k
EGDALPNPR	325-333	11.8	I-A^k
QNFVMAPAQ	347-355	9.5	I-A^k
FVMAPAQAA	349-357	20.2	I-A^b
DAQAPQAPA	381-389	20.65	I-A^k
AATTTAQTS	357-365	12.8	I-A^b
AATATVRVA	369-377	13.04	I-A^b
NGDTTENKT	415-423	12.5	I-A^k
DGFMPKIVL	437-445	14.7	I-A^k
FDKTWGQNS	448-456	16.3	I-A^b
DNSVNEISP	523-531	11.6	I-A^k
FYITVMMKA	536-545	12.6	I-A^b
HKELHLQQR	550-558	9.6	I-A^k

[a]higher scores represent high affinity binding to MHC class II molecules.

(Noormohammadi *et al.*, 2002). Since this deletion was not observed in the VlhA *M. synoviae* WUV1853 strain, we speculate that the *M. synoviae* WUV1853 strain may be more virulent than the *M. synoviae* 53 strain.

Identification of immunodominant epitopes within a vaccine candidate antigen is extremely useful, since it is possible to formulate a vaccine composed of relevant epitopes from different antigens. *In silico* epitope predictions resulted in the identification of 17 B cell epitopes, ranging from 7 to 23 mers (Table 1), and 22 T cell epitopes of 9 mers (Table 2). The predicted epitopes were distributed along the entire protein sequence. Comparing the epitopes predicted from the VlhA protein sequence of the *M. synoviae* 53 strain with the *M. synoviae* WUV1853 strain, we observed that 13 of them are shared by both strains as indicated in Figure 1. Additionally, amino acids from S_{195} to L_{211}, K_{345} to A_{356}, and N_{440} to E_{446} represent epitopes that could be recognized by either T and B cells, as shown in Figure 1. These B and T cells epitopes can improve efficacy of these peptides in vaccine design.

B and T cell epitopes comprised in the VlhA protein and shared by *M. synoviae* 53 and *M. synoviae* WUV1853 strains were also compared to sequences of other *Mycoplasma synoviae* and *Mycoplasma gallisepticum* strains. These analyses revealed that the B cell epitope S_{263}-D_{277} and the T cell epitopes N_{45}-N_{54} and G_{58}-N_{67} showed 100% and 87-100% identity, respectively, with corresponding regions of the VlhA protein of *Mycoplasma synoviae* and *Mycoplasma gallisepticum* strains (Table 3 and 4).

Although *in vivo* or *in vitro* assays have to be performed to confirm these selected peptides, *in silico* epitope prediction has been used in many studies in the development of new immunodiagnostic and vaccine formulations (Panigada *et al.*, 2002; Iwai *et al.*, 2003; Fonseca *et al.*, 2004). Identification of T and B cell epitopes on different *Mycoplasma* strains become even more relevant since evasion mechanisms used by these bacteria to escape host immune response are based on antigen mimicry and antigenic variability (Markham *et al.*, 1994; Wren, 2000). Among B cell predicted epitopes, peptides E_{94}-E_{101}, T_{165}-L_{171}, S_{195}-L_{211}, A_{220}-A_{237}, S_{263}-D_{277}, K_{345}-A_{356}, T_{364}-V_{376} and M_{440}-E_{446} are shared between VhlA of *M. synoviae* 53 and *M. synoviae* WUV1853 strains (Figure 1). The S_{263}-D_{277} peptides represent the most conserved B cell epitope which possesses 100% identity with peptides of the VhlA from the *M. synoviae* K1968, MS-H, ULB925 and ULB925KF strains and also with *M. gallisepticum* S6 and R strains (Table 3).

Regarding T cells epitopes, N_{45}-N_{54} and G_{58}-N_{67} are the most conserved epitopes (with identity ranging from 87-100%) found in the VhlA of the majority of *M. synoviae* strains tested and also within the *M. gallisepticum* S6 strain (Table 4). Finally, the analysis performed here demon-

Table 3 - *Mycoplasma synoviae* 53 B cell epitopes identity with other mycoplasma strains and species.

Strain	GenBank accession number	E_{94} -E_{101}	T_{165}-L_{171}	S_{195}-L_{211}	A_{220}-A_{237}	S_{263}-D_{277}	K_{345}-A_{356}	T_{364}-V_{376}	M_{440}-E_{446}
M. synoviae K1	CAE45740	100%	—	—	—	—	—	—	—
M. synoviae K1968	AF314230.1	—	—	100%	82%	100%	—	—	—
M. synoviae K2581	AAG48110	—	—	100%	77%	—	—	—	—
M. synoviae K27	KAE46393	100%	—	—	—	—	—	—	—
M. synoviae MS-H	AF464936.1	—	100%	94%	88%	100%	91%	91%	—
M. synoviae TN/427	AAX84496	—	100%	—	—	—	—	—	—
M synoviae ULB925	AF488712.1	—	—	94%	76%	100%	—	—	—
M. synoviae ULB925KF	AF314228.1	—	—	94%	77%	100%	—	—	—
M.gallisepticum R	NB853210	—	100%	88%	83%	100%	100%	65%	85%
M gallisepticum S6	AAB 50153	—	—	—	—	100%	100%	69%	85%

Table 4 - *Mycoplasma synoviae* 53 predicted T cell epitopes identity with other mycoplasma strains and species.

Strains	GenBank accession number	N_{45}-N_{53}	G_{58}-N_{66}	D_{201}-P_{209}	Y_{289}-N_{297}	E_{325}-R_{333}	F_{349}-A_{357}	N_{415}-T_{423}	D_{437}-L_{445}
M. synoviae B133-96	CAE45733	100%	100%	—	—	—	—	—	—
M. synoviae B154-02	CAE45738	100%	100%	—	—	—	—	—	—
M. synoviae B2700	CAE45735	100%	100%	—	—	—	—	—	—
M. synoviae B31-88	CAE45737	90%	87%	—	—	—	—	—	—
M. synoviae B38-96-170	CAE45732	100%	100%	—	—	—	—	—	—
M. synoviae B94-91	CAE45739	87%	—	—	—	—	—	—	—
M. synoviae J26-85	CAE45729	90%	87%	—	—	—	—	—	—
M. synoviae J151-85	CAE45728	90%	87%	—	—	—	—	—	—
M. synoviae K1	CAE45740	100%	100%	—	—	—	—	—	—
M. synoviae K4	CAE45731	100%	100%	—	—	—	—	—	—
M. synoviae K1968	AF314230	100%	100%	100%	90%	100%	—	—	—
M. synoviae K2581	AAG48110	100%	100%	100%	80%	—	—	—	—
M. synoviae K27	CAE46393	100%	100%	—	—	—	—	—	—
M. synoviae MS-H	AF464936.1	100%	100%	90%	90%	100%	90%	—	90%
M. synoviae TN/427	AAX84496	100%	100%	—	—	—	—	—	—
M. synoviae ULB925	AF488712.1	90%	87%	90%	80%	100%	88%	80%	100%
M. synoviae ULB925KF	AF314228.1	90%	87%	90%	80%	90%	—	—	—
M.gallisepticum R	NB853210	—	—	100%	80%	100%	100%	90%	100%
M. gallisepticum S6	AAB50153	100%	100%	—	—	100%	100%	—	100%

strated that there are conserved B and T cell epitopes mainly in the N-terminal region of the VhlA protein from the *M. synoviae* 53 strain, which may represent potential targets for the development of new diagnostic assays and subunit vaccines.

Acknowledgements

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Abbreviations

Vlha: Variably expressed lipoprotein and hemagglutinin.
PRR: Protein rich region.
MSPB: Major surface protein B.
MSPA: Major surface protein A.
PSSMS: Position specific scanning matrices.
HA: Hemagglutination.

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Internet Resources

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