



Phenotypic and antigenic characterization of *Mycoplasma gallisepticum* by RFLP and SDS-PAGE

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The most economically important mycoplasma species in poultry is *Mycoplasma gallisepticum* (MG). There is a great variability between strains in respect of tissue tropism (proclivity for brain, eye, joint), culturing ability, virulence and transmissibility and antigen structure. It is an important respiratory tract and systemic pathogen of commercial poultry and is regarded as an economically significant cause of disease confronting.

Several techniques aimed at differentiating the vaccine F strain from wild-type strains were developed. These include SDS-polyacrylamide gel electrophoresis of total proteins (Khan *et al.* 1987a), restriction endonuclease analysis of genomic DNA (Kleven *et al.* 1988), dot blot hybridization with a specific DNA probe for the vaccine F strain (Khan *et al.* 1987b), and polymerase chain reaction with specific oligonucleotides for the strain (Nascimento *et al.* 1993). These are useful for identification of the vaccine strain, even though they have not yet been fully applied to the taxonomic classification of other MG strains.

The objective of this study was to search protein pattern and restriction endonuclease analysis that could be used to distinguish it from vaccine strain MG 6/85 and field isolates.

Micro-organisms: Live *Mycoplasma gallisepticum* vaccine- 6/85 stored in the Department of Veterinary Microbiology, Veterinary College and Research Institute, Namakkal was used as reference culture. The 4 strains (MG1, MG2, MG3, and MG4) were isolated from 4 naturally infected flocks; 2 were recovered from sinus cavity and 2 from air sacs.

Hyperimmune serum: Reference *Mycoplasma gallisepticum* hyperimmune serum maintained in the Department of Veterinary Microbiology, Veterinary College

and Research Institute, Namakkal, was used.

Cellular antigen preparation and PCR: For testing mycoplasma strains an aliquot (1.0 ml) of each culture was centrifuged at 14,000 rpm; pellet washed with PBS (pH7.2), resuspended in 20 µl nuclease free water and boiled for 5–10 min and then kept in ice. DNA amplifications were performed as per Kiss *et al.* (1997).

RFLP analysis: Two restriction endonucleases - *Bam*H1 and *Eco*R1 with buffers were used according to the manufacturer's recommendations to digest the PCR amplicons. This digestion mixture (MG1, MG2, MG3, MG4 and MG- 6/85 vaccine) was incubated for 3 h at 37°C in water bath. After the incubation period the reaction was stopped by heating at 65°C for 30 min. Restriction patterns were observed using agarose gel electrophoresis and carried out on a 2% agarose gel containing ethidium bromide (0.5 µg/ml) at a constant 100 volts for 45 min.

SDS-PAGE analysis: The mycoplasmal proteins were submitted to SDS-PAGE electrophoresis with 10% (w/v) of acrylamide/bis 37.5% gels and stained with Coomassie brilliant blue in 25% methanol: 10% glacial acetic acid, destained overnight with 2 changes of 25% methanol: 10% glacial acetic acid, and further destained for 2 h with 10% glacial acetic acid: 1% ethanol. Photographs were taken by using polaroid camera.

Haemagglutination inhibition test: Haemagglutination inhibition assay to detect Mg antibodies were performed on hyperimmune serum using 4 haemagglutination units of antigen obtained from MG 6/85 (Kleven and Yoder Jr 1989). Positive and negative MG stand sera were used as controls.

This study describes a rapid and reliable technique for the differentiation of MG isolates/strains. Increasing use of the MG live vaccines in poultry has led to a need for a reliable technique that can differentiate MG vaccine strains from field isolates. This is primarily for epidemiological investigation, but may also be required by registration authorities when a new MG vaccine is introduced to a country.

All the 4 field and vaccine isolates were subjected to

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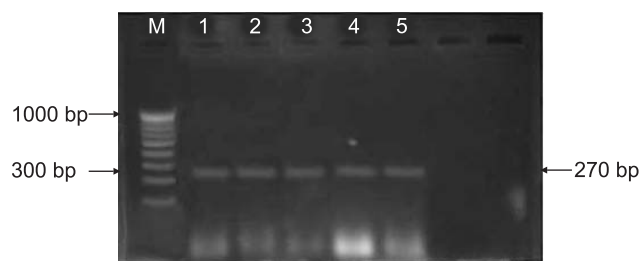


Fig. 1. M – 100 bp Molecular weight marker; lane 5, MG vaccine strain (6/85). Lane 1, 2, 3 and 4 – MG field isolates.

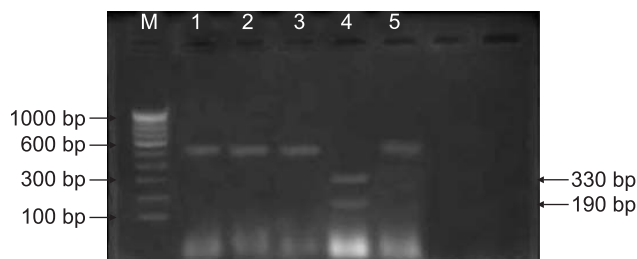


Fig. 2. M – 100 bp molecular weight marker; lane 5 – MG vaccine strain (6/85). Lane 1, 2, 3 and 4 – MG field isolates.

nucleic acid amplification of 16s rRNA *Mycoplasma gallisepticum* genome by polymerase chain reaction (PCR) were produced predicted size of 530 bp amplicons in the PCR reaction. This observation was in agreement with results of Kiss *et al.* (1997).

In the present study, the region of the 16s rRNA gene of the field and vaccine isolates was amplified and the PCR products were digested with *Bam* H1 and *Eco* R1 restriction enzymes. After digestion with *Bam* H1, one band of size approximately 270 bp (Fig. 1) could be appreciated in all the 4 field and vaccine isolates. This is in agreement with Kiss *et al.* (1997). After digestion with *Eco* R1, two bands of sizes approximately 330 bp and 190 bp (Fig. 2) could be appreciated only in 1 field isolate. The DNA cleavage patterns of field MG isolates and the reference strain (6/85) appeared identical to each other except the MG4 isolate. This could be due to the strain variation. This needs further investigation.

Phenotypic variation among MG field isolates and reference strain were better detected by SDS-PAGE (Fig. 3). Ley *et al.* (1993) reported that both SDS-PAGE and REA were capable of discriminating among S6, R and F strains and both indicated that the field isolates tested were identical or nearly identical to each other and to F strain. In the present study fractionation of proteins of the field isolates and the referral strain (MG 6/85) showed 6 polypeptides. The molecular weights of different polypeptides of the isolates were 25kDa, 43 kDa, 68 kDa, 84 kDa, 97 kDa and 205 kDa. These findings are in correlation with the findings of Khan *et al.* (1987b) and Ferraz *et al.* (2000). We also described that the major difference among isolates tested was the detection of a 150 kDa band exclusively in MG4 but not in other field isolates and in MG-6/85. These results confirmed the previously reported phenotypic diversity between F and S6 MG strains

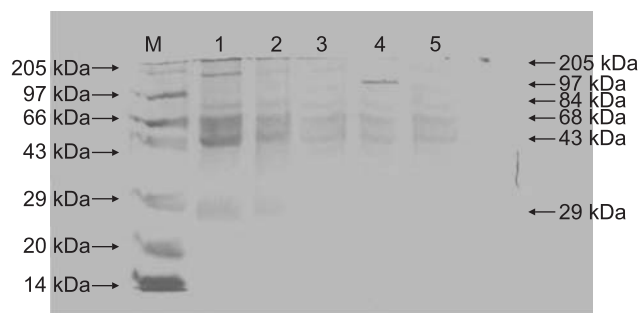


Fig. 3. M – 3kDa to 205kDa molecular weight marker; lane 5 – MG vaccine strain (6/85). Lane 1, 2, 3 and 4 – MG field isolates.

(Khan *et al.* 1987b, Thomas and Sharp 1988).

All the field and vaccine MG isolates were further subjected to haemagglutination inhibition (HI) using hyperimmune serum. Hyperimmune serum gave HI titre of 80 for all the field and vaccine isolates. When hyperimmune serum response to the MG isolates was evaluated by haemagglutination, no antigenic variability was detected when comparing the vaccine and field isolates. Previous report showed that the HI assay is not suitable to distinguish variability among strains responsible for infection and serum conversion (Thomas and Sharp 1988).

The SDS-PAGE and RFLP techniques provide a rapid and reliable means for detection and differentiation of MG strains from both culture and clinical swabs and it would be a useful procedure in epidemiologic and other studies where minor but distinctive differences in protein profile and DNA cleavage patterns may be used to identify a particular strain of MG. This illustrates the discriminatory power of SDS-PAGE and RFLP analysis for the differentiation of MG 6/85 from other MG strains. The occurrence of clinical infection in layer caused by MG underscores the importance of rigorous biosecurity on the part of layer industry to eliminate or curtail the potential for exposure of MG. Furthermore, these findings place added prominence on the need for effective MG vaccines, designed for use in poultry industry.

SUMMARY

Phenotypic and antigenic variations among *Mycoplasma gallisepticum* vaccine 6/85 and field isolates were investigated by SDS-PAGE, RFLP and haemagglutination inhibition test. The SDS-PAGE system followed by photographic analysis showed weak variability between isolates, being the major difference close to the 150 kDa level where a prominent peptide band was detected only in the MG4 isolate. After digestion with *Eco* R1, two bands of sizes approximately 330 bp and 190 bp could be appreciated only in MG4 field isolate. Hyperimmune serum was used in serological test for this antigenic variability study. There were strong cross reaction between isolates. These studies verified the utility of newer technologies for disease outbreak investigation and the possibility of MG causing disease in layers under the field condition.

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