Introduction

The *Mycoplasma gallisepticum* (MG) infection of chickens and turkeys causes considerable economic losses because of deficiency of Mycoplasma control and increasing spread of variant strains. Beside this the usage of MG live vaccines has led to a need for differentiation of MG strains.

Beside MG and other avian mycoplasmas *M. imitans* (MI) can be isolated from chickens. MI has phenotypic properties very similar to MG properties, but the molecular properties differ from each other. The serological cross-reaction can cause diagnostic difficulties. Beside serological examination and Mycoplasma culturing the molecular methods take an important place in diagnostic investigations.

The differentiation of Mycoplasma strains based on DNA patterns. The PCR-RFLP methods specific for the genes take place in Mycoplasma pathogenesis can be able to distinguish MG live vaccine strains or MG strains isolated from chickens or turkeys. The discrimination of the different MG strains can be useful for epidemiological investigation of infection.

Goals of the study:

1. Comparison of MI and MG strains using RAPD-PCR methods.

2. Comparison of some gene sequences of MI and MG strains using PCR-RFLP methods.

3. Sequencing comparison of some PCR amplicons.

4. Comparison of MI and MG strains based on multivariate statistical methods.

Materials and methods

The mycoplasma strains

Twelve MG strains with different properties and origin was used:

- MG F and MG TS-11 live vaccine strains.
- MG MK-7 (pathogen) and MG MS-16 (non-pathogen) isolates from Japan.
- MG FS-9 (1226) chicken isolates from Hungary.
- MG X-95 and MG S6 isolates.
- MG Rlow pathogen strains and its non-pathogen clones (MG Rhigh, MG Rhighm, MG RCl, MG M3).

Beside MG strains MI 4229 referent strain was used too.

The basis of Mycoplasma differentiation

After culturing the Mycoplasma DNA was prepared. Two previously described RAPD PCR (Fan *et al.* 1995; Charlton *et al.* 1999) and PCR methods specific for *recA*, *crmA*, *crmB*, *crmC*, *gapA*, *mgc2*, *LP* and *pvpA* genes was performed. The *recA* gene plays an important role in DNA repair, the *pvpA* gene encoding haemagglutinin, the other genes encoding adhesin-like proteins, which take place in MG pathogenesis. We examined the differentiating potential of PCR primers described previously or performed in this study targeted to these genes. The PCR amplicons were digested restriction endonucleases. Based on PCR-RFLP patterns multivariate statistical methods were used. Beside this some of the PCR amplicons were sequenced and compared with each other.

Results

1. Results of RAPD PCR

Both of the two RAPD PCR methods the Mycoplasma strains were classified into 6 clusters. However the members of the clusters were different using the two methods. Using Fan *et al.* (1995) RAPD PCR method the MI 4229, the MG F and the MG TS-11 vaccine strains could be distinguished. Usage Charlton *et al.* (1999) primers it was not possible.

2. Results of PCR-RFLP specific for selected genes

a.) Results of PCR-RFLP specific for *recA* gene
Using primers previously described the differentiation of Mycoplasma strains was not possible and MI 4229 was amplified. While we used primers performed in this study we could not detected MI 4229 and the strains were classified into two classes.

b.) Results of PCR-RFLP specific for *crmA* gene
For characterization of *crmA* gene five PCR methods were performed. Two variable sequences were identified in the gene. Based on these variable regions the strains could be distinguished. Beside this partial sequences of the gene could not detected by the MI 4229, MG MK-7, MG S6, MG MS-16 strains and the MG F vaccine strain.

c.) Results of PCR-RFLP specific for *crmB* gene
Based on four PCR and RFLP performed for gene characterization difference among the strains was not shown. Whole of the gene was not amplified by MI 4229 and MG F vaccine strain.

d.) Results of PCR-RFLP specific for *crmC* gene

For examination of *crmC* gene five PCR methods were developed. After the RFLP analysis three parts of the gene proved to be variable. However based on PCR-RFLP the MG TS-11 vaccine strain can distinguished from other MG and MI 4229 strains.

e.) Results of PCR-RFLP specific for *gapA* gene

Beside previously described primers six PCR methods were developed for characterization of the *gapA* gene. We founded a sequence on which the MI 4229, the MG F and MG TS-11 vaccine strains can differentiate from each other and from the other MG strains. Based on the results this PCR-RFLP method can used in diagnostic way.

f.) Results of PCR-RFLP specific for *mgc2* gene

Because of variability of the *mgc2* gene the PCR-RFLP method developed in this study proved to be suitable for distinguish the MG and MI 4229 strains and use in diagnostic methods.

g.) Results of PCR-RFLP specific for *LP* gene

Based on the previously described PCR method specific for *LP* gene and the RFLP patterns developed in this study there were not differences among the strains.

h.) Results of PCR-RFLP specific for *pvpA* gene

Using previously described PCR-RFLP methods the strains were divided into several groups. However the MG TS-11 vaccine strain can not distinguish from other strains.

3. Results of sequence analysis

Sequence analysis was performed on the *recA*, the *crmA*, the *crmC*, the *gapA*, the *mgc2* and the *pvpA* genes. For the analysis the MG TS-11, the MG Rhigh, the MG X-95 and the MG M3 strains were used. Based on the gene sequences the *crmA*, the *gapA* and the *mgc2* genes proved to be variable.

New results

The previously described PCR-RFLP methods or PCR-RFLP patterns performed in this study proved to be suitable to differentiate pathogen and non-pathogen MG an MI strains isolated from chickens, turkeys or wild birds. Based on the results the MG live vaccine strains can be identified.

On the results of the study

1. Based on the ***gapA*****, the *mgc2* and the *pvpA*** genes differences were found among the **MI 4229 strain and the other MG strains.**
2. Based on the ***crmC*** **and the *gapA* genes the MG TS-11 vaccine strain** can distinguished **from the other MG and the MI 4229 strains.**
3. Based on the ***gapA*** **gene the MG F vaccine strain** can distinguished **from the other MG and the MI 4229 strains.**
4. **The results can be useful for epidemiological investigation of infection.**
5. The partial sequences of the ***recA*****, *crmA, crmC, gapA*****, *pvpA* and *mgc2* genes by MG Rhigh and MG M3 strains were described**.
6. The partial sequences of the ***recA*****, *crmA, crmC, gapA* and *mgc2* genes by MG TS-11 strain were described**.
7. The partial sequences of the ***crmA, crmC, pvpA*** **and *mgc2* genes by MG X-95 strain were described**.

List of publications

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