PCR Detection and differentiation of Mycoplasma gallisepticum field samples from 6/85.

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INTRODUCTION

Mycoplasma gallisepticum (Mg) is a pathogenic agent, which causes important financial losses each year. Mg causes a Chronic Respiratory Disease (CRD) in chickens and sinusitis in turkeys. At present, there is a great interest in typing vaccines and wild type strains of Mg. Types of vaccines to be differentiated vary depending on the country and the vaccination programs. The goal of this study was the identification and differentiation of the 6/85 vaccine strain.

Up to now, the study of an unique gene has not been enough to carry out the characterization of Mycoplasma strains. Currently, other significant genes such as RNAr 16S, Lp, gapA, pvpA or Mgc2 are being studied. In order to differentiate field isolates from the 6/85 vaccine, INGENASA has developed two PCR methods based on the Lipoprotein (Lp) and the Cytoadhesing (Mgc2) protein genes .

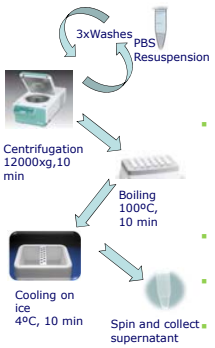
MATHERIALS & METHODS

Obtaining the DNA

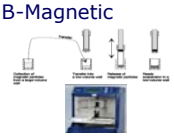
Sampling

Extraction

A-Boiled

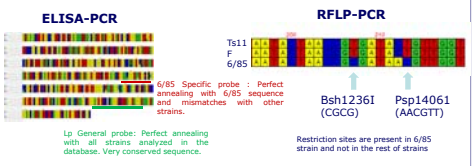


B-Magnetic



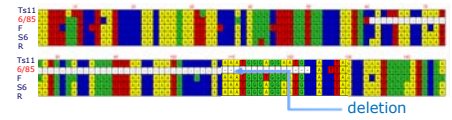
- Sample is mixed with binding buffer and mechanically disrupted with zirconia beads during 15 minutes to release DNA.
- Magnetic beads with nucleic acid affinity capture the DNA.
- The magnet capture the beads with the DNA.
- After 4 washing steps, DNA is eluted in small volume.

Lp primers for Detection and Sequence analysis



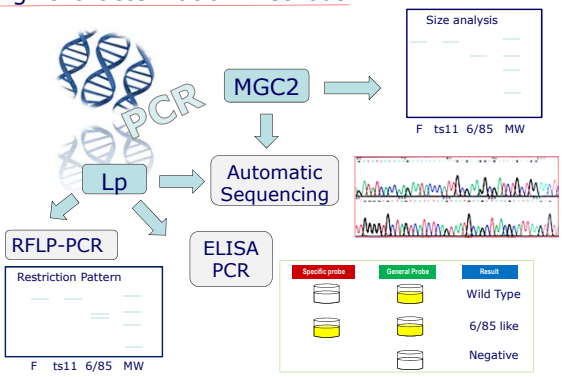
Lp primers were selected for high sensitivity and specificity to detect Mg samples without previous growing. They allowed typing different patterns by probes, RFLP digestion and sequencing.

Mgc2 primers for 6/85 DNA Size Differentiation



Mgc2 primers were selected close to a deletion in the 6/85 strain being suitable to be differentiated by agarose gel migration.

Mg characterization methods



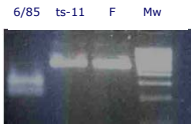
- PCR conditions: 95ºC 5 minutes, followed by 35 cycles (94ºC 1 minute, 50ºC 1 minute and 72ºC 1 minute) and a final extension step for 7 minutes at 72ºC.
- Lp PCR products were digested with 10 units of Bsh1236I or Psp1460I (Fermentas) , at 37ºC for 2h or analyzed by ELISA or automatic sequencing.
- Mgc2 PCR products were fragment length analyzed by agarose gel electrophoresis and automatic sequencing.

RESULTS

Lp PCR Results

A) Amplification

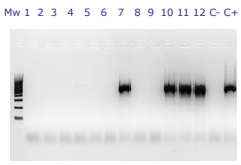
B) RFLP (Bsh 1236I)



C) ELISA-PCR

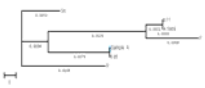
Id Sample	Mg Lp General	MG Lp 6/85
Vaccine	1.11	1.06
Wild Type	0.735	0.053
ts 11	1.99	0.182
6/85	2.54	1.102
F	2.57	0.056

A_1) Specify



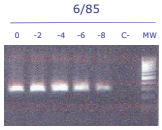
1-Mycoplasma meliagridis
2-Mycoplasma imitants
3-Mycoplasma iowae
4-Acoleplasma laidlawii
5-Mycoplasma gallinaceum
6-Mycoplasma gallinarum
7-Mycoplasma gallisepticum –Cepa R
8-Mycoplasma synoviae –WVU 1853
9-Mycoplasma synoviae –vacuna MS-H
10-Mycoplasma gallisepticum -ts-11
11-Mycoplasma gallisepticum – cepa F
12-Mycoplasma gallisepticum -6/85

D)Sequencing 6/85



- Using these methods strains with a 99% of homology with 6/85 were detected.

A_2) Sensitivity



5 UFC/ml (dilutions of C+)

- The Lp PCR was tested in a interlaboratory assay.
- Samples were provided by the reference laboratory (University of Georgia, Department of Population Health).
- Specificity and sensitivity of the assay were 100%.

E) Field Results

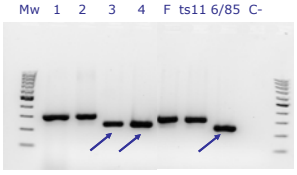
Results	Number of Samples	Percentage %
Total	1462	100
Positives	271	18.53
Negatives [a]	1191	81.47

a)Sample from breeder farms were negative.

Mgc2 PCR Results

A) Vaccines

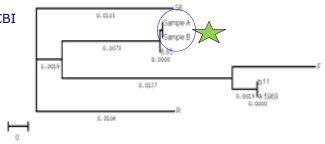
B) Field Samples



Phylogenetic Trees and BLAST analysis of field samples (A and B)

1) Lp sequences analysis

6/85 strain was the first option when sequences were blasted with the NCBI database, for both A and B samples.



2) Mgc2 sequences analysis

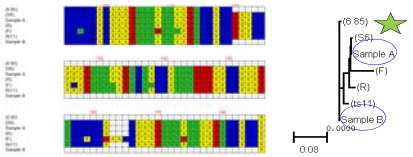
Distance to 6/85 was different for samples A and B. First option, when blasted with the NCBI databases, was 6/85 in the case of sample B, but not for sample A. This result correlated perfectly with the size analysis for Mgc2.

3) Typing

Samples could be divided into two groups:

a) Field strains showing the same genetic sequences as the 6/85 vaccine in the Lp gene, but different for the Mgc2 gene → field sample A.

b) Other strains identical to 6/85 for both genes → vaccine, sample B.



DISCUSSION

- In order to differentiate the 6/85 vaccine, two genes of *Mycoplasma gallisepticum* that have been related to respiratory tract invasion, have been studied: Lipoprotein gene (Lp) and Cytoadhesin gene (Mgc2).
- Several molecular methods and sequencing were used:
  - Lp analysis was made by RFLP-PCR, specific probes ELISA-PCR and automatic sequencing.
  - Mgc2 gen was tested by fragment length analysis on agarose gel and automatic sequencing.
- Ring test trial for Lp detection showed excellent results in relation with sensitivity and specificity. Nevertheless, the method was not good enough to differenciate 6/85 vaccine from field isolates, therefore Mgc2 gene analysis was necessary to type 6/85 vaccine.
- Concerning Spanish situation, the obtained results suggest that the described Mgc2 length analysis of the 6/85 fragment, is the best option to differenciate vaccinated animals, being very useful to follow up the state of farms.