DETECTION OF MCS4 RNA GENES IN *MYCOPLASMA CAPRICOLUM* SUBSP. *CAPRICOLUM* TYPE STRAIN CALIFORNIA KID AND STRAIN GM262G

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Summary. MCS4 RNA (125 nucleotides in length) is as abundant 5S rRNA in the cell and is encoded by two genes – *mcs4a* and *mcs4b*. We detected MCS4 RNA genes by random amplified polymorphic analysis (RAPD) and Southern hybridization technique. In our studies the genomic polymorphisms of *Mycoplasma capricolum* subsp. *capricolum* type strain California kid (ATCC 27343) and strain GM262G (ATCC 43092) were not detected by RAPD analysis with the pure DNA and with amplified products of these strains.

Results of Southern hybridization with genomes of California kid and GM262G using two hybridization probes *mcs4a* US–C1 and *mcs4b* US–N2, have showed that signals were detected for the genome California kid using both probes. The strain GM262G has only gene *mcs4*b and the signal was detected only with the probe *mcs4b* US-N2.

Keywords: *Mycoplasma capricolum,* genes, goats, MCS4 RNA

MCS4 RNR GENŲ NUSTATYMAS *MYCOPLASMA CAPRICOLUM* PORŪŠIO *CAPRICOLUM* ETALONINĖJE PADERMĖJE CALIFORNIA KID IR PADERMĖJE GM262G

Santrauka. MCS4 RNR (ilgis 125 nukleotidai) yra gausu 5S rRNR ląstelėse. MCS4 RNR randami du genai – *mcs4a* ir *mcs4b.* Taikydami atsitiktinės amplifikuotos polimorfinės DNR (AAPD) ir Southern hibridizacijos metodą, tyrimais norėjome nustatyti MCS4 RNR genus. Genetinio polimorfizmo AAPD metodu etaloninės padermės California kid (ATCC 27343) ir padermės GM262G (ATCC 43092) genetinėje medžiagoje ir amplifikuotame produkte nenustatėme.

Southern hibridizacijos metodu genome California kid ir GM262G, pagal pavyzdžius *mcs4a* US–C1 ir *mcs4b* US–N2 signalus nustatėme California kid genome su *mcs4a* ir *mcs4b*.

Kadangi padermė GM262G turi tik vieną geną – *mcs4b*, signalas buvo nustatytas tik su *mcs4b* US–N2.

Raktažodžiai: *Mycoplasma capricolum*, genas, ožkos, MCS4 RNR.

Introduction. Mycoplasmas are small wall-less microorganisms, which are phylogenetically related to gram-positive bacteria from *Bacillus and Clostridium* genus (Weisburg et al., 1989). They are parasitic in eukaryotic tissues and organs, and their genomes are the smallest in all self-replicating organisms (Himmelreich, 1996). *Mycoplasma capricolum* is parasitic in goats. Its genome is about 600 –1,100 kbp in length (Whitley et al., 1991, Bove, 1993, Bork et al., 1995). The genome contains only two sets of rRNA (Sawada et al., 1981, Sawada et al., 1984), 30 genes for 29 tRNR species (Andachi et al., 1989). There are at least six small stable RNAs in *M.capricolum* cells besides tRNAs and rRNAs (Ushida and Muto, 1995). Among them, MCS1, MCS5 and MCS6 are the homologs of the *Escherichia coli* 4.5S RNA, M1 RNA (RNase P RNA), and 10Sa RNA (tm RNA), respectively (Ushida et al., 1996).

MCS4 RNA (125 nucleotides in length) is as abundant 5S rRNA in the cell and is encoded by two genes – *mcs4a* and *mcs4b*. There is a special interest on MCS4 RNA sequence for its similarity to eukaryotic U6 snRNAs (Ushida and Muto, 1993).

Only limited species of mycoplasma belonging to the *Mycoplasma mycoides* cluster have MCR4 RNA genes.

Figure 1 shows that four strains of *M.capricolum* subsp. *capricolum* have both genes *mcs4a* and *mcs4b*. The strain GM262G has only *mcs4b*.

Calcutt et al. (2002) reported since *mcs4a* is on the conjugative element, this may move among genomes of the *M. capricolum* subsp. *capricolum* strains. In contrary, *mcs4b* may not be the gene of the conjugate element and stable exists in the genome of the *M. capricolum* subsp. *capricolum* strains.

Random amplified polymorphic DNA (RAPD) analysis is an easy and fast method to detect genomic polymorphisms (Stakenborg, 2002). By this technique expected to detect the difference between the genomes of type strain California kid, strain GM262G and to determine *mcs4a* and *mcs4b* by Southern hybridization.

Materials and methods. *Mycoplasma capricolum* subsp. *capricolum*, type strain California kid (ATCC 27343) and strain GM262G (ATCC 43092) (American Type Culture Collection) were grown in modified Edward medium (MEM) at 37 °C (Razin and Rottem, 1976). Cells were harvested at late-long phase by centrifugation at 7.000 rpm for 40 min at 4 °C, washed with TMN – buffer and stored at – 20 °C. Crude preparation of DNR was obtained by the direct phenol extraction method (Ausubel et al., 1995).

Random amplified polymorphic DNA (RAPD) was obtained with the pure DNA and amplified products of California kid, GM262G using six different single oligonucleotides of arbitrary sequences (7– 15 bases in length) (Amersham Biosciences). Control DNA and primes were performed by using two *Escherichia coli* strains BL21 (DE3), C1a DNAs.

The PCR (Perkin Elmer Geneamp PCR system 2400) was carried out under low stringency conditions to generate a reproducible array of strain specific products.

The amplified products were analyzed by using gel electrophoresis, using 2% agarose gel (Agarose MP, Multi purpose agarose, Boehringer Manheim) containing ethidium bromide (0.5 μg/ml) for 1.5 hours at a constant 50V, in 1x TAE buffer. A molecular 100-bp ladder DNA size marker was run concurrently. The thermocycler conditions consisted of an initial dena-turation at 95 °C for 5 min, followed by 45 cycles at 95 °C for 1 min, 40 °C for 1 min, finally – 72 °C for 2 min.

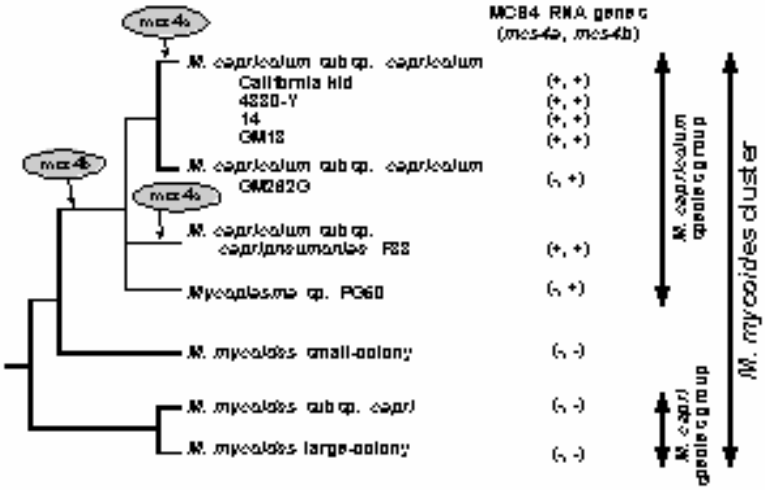

Figure 1. **Distribution of MCS4 RNA genes in the species belongs to the *Mycoplasma mycoides* cluster. The schematic tree of the species in the cluster is based on Pettersoon et al. (1996). The presence (+) and (or) absence (–) of *mcs4a* and *mcs4b* are indicated in parentheses.**

Southern blot and hybridization protocols were used for detection of *mcs4a* and *mcs4b* on the *genome* of California kid and GM262G. DNA of California kid and GM262G were restricted by using enzymes *Eco*RI and *Hind*III (Gibc BRL). DNA of these strains were separated by 1% agarose gel electrophoresis and transferred to a positively charged nylon membrane (Hybond – N^+ Amresham). The oligonucleotides (5' – TATTAAGTCA TTAACCGGTT-3') and (5'TGCCTTTAAACAAGG CTGT'3) that have a complementary sequence to a part of the MCS4 RNA were labelled with Gene Images Alk Phos Direct labelling and detection system from Amersham Pharmacia Biotech. Hybridization and detec-tion were performed with some modifications of manufacturer's procedures: the membrane was incubated with the probes at 55 °C for 12h and then washed in x 2 SSPE at 60 °C for 10 min twice, 2x SSPE at room temperature for 5 min twice. The washed membrane was exposed to Fuji RX films for 1 h.

Results. Random amplified polymorphic DNA (RAPD) analysis firstly was obtained using two control *E. coli* strains BL21 (DE3), C1a DNAs and RAPD analysis six primers. After analysis of control DNA RAPD reactions on 2% agarose gel we obtained polymorphism of DNA banding patterns between two strains of *E.coli.* However, by RAPD analysis repro-ducible array with pure DNA of California kid and GM262G using six different single oligonucleotides of arbitrary sequences were not constantly obtained. Only with primer 4, (Figure 2) we have obtained reproducible array of California kid and GM262G. Different loci of DNA banding patterns between these two strains were not detected.

Figure 2. **Electrophoresis of RAPD analysis (annealing 40° C) products *of Mycoplasma capricolum* subsp. *capricolum* GM262G (G) 0.02 μg) California kid (C)**

Figure 3 A and Figure 3 B show the results of the Southern hybridization with the genomes of the type strain California kid and strain GM262G. Oligonucleotide *mcs4a* US-C1 has sequences corresponding to the upstream sequences of the MCS4 RNA coding region *mcs4a*. The, other, designated *mcs4b*-US-N2 has a sequence corresponding to the upstream sequence of MSC4 RNA coding region of *mcs4b*. Signals were detected for the genomes of the type strain California kid with the probes *mcs4a* US-C1 and *mcs4b* US-N2 (Figure

3A). However, only signal with the probe *mc4b* US-N2 was detected of the strain GM262G (Figure 3B).

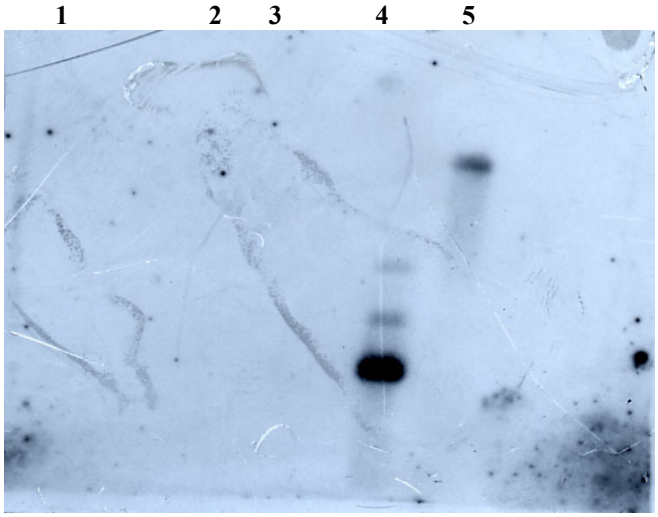

Figure 3A. **Detection of *mcs4a* on the genome California kid and GM262G by Southern hybridization**
lane 1 - GM262G digested with *EcoRI*
lane 2 - GM262G digested with *Hind*III
lane 3 - empty
lane 4 - California kid digested with *EcoRI*
lane 5 - California kid digested with *Hind*III

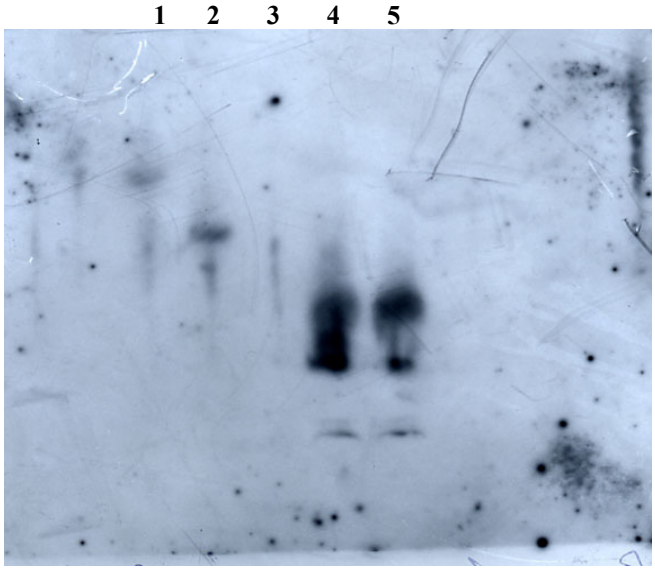

Figure 3B. **Detection of *mcs4b* on the genome California kid and GM262G by Southern hybridization**
Lane 1 - GM262 G digested with *Eco RI*
Lane 2 - GM262G digested with *HindIII*
Lane 3 - empty
Lane 4 - California kid digested with *Eco RI*
Lane 5 - California kid digested with *Hind* III

Southern hybridization was used and with amplified products of California kid and GM262G. The signals were not detected with both probes on amplified products of California kid and GM262G.

Discussion. According to the phylogenetic tree of mycoplasmas belonging to the *Mycoplasma mycoides* cluster, only *M.capricolum* subsp. *capricolum* strains have MCS4 RNA genes. The gene *mcs4b* exists in all strains of *M. capricolum* subsp. *capricolum*. However, the *mcs4a* are absent in strain GM262G. Our investigations by Southern hybridization consent to the date of Dr.Uschida et.al. (In press), that GM262G has only *mcs4b* genes.

Calcutt et al. (2002) reported that *mcs4a* of strain California kid is included in a conjugative element (ICEC), which exists in an extrachromosomal form, and moves with ICEC among mycoplasmas of the *M .capricolum* subsp. *capricolum* strains. *Mcs4b* may not have gene of the conjugative element and permanently exists in the genome of the *M. capricolum* subsp. *Capri-*

colum strains (Moran and Chandler, 2002; Van der Meer and Sentchilo, 2003).

We used Random amplified polymorphic DNA (RAPD) analysis for detection genomic polymorphisms between type strain California kid and strain GM262G. However, it is likely that genomic analysis by RAPD technique does not help elucidate polymorphism between two above-mentioned strains.

Conclusions. In our studies the genomic polymorphisms between type strain California kid and strain GM262G were not detected by RAPD analysis.

The results have shown that using Southern hybridization with amplified products of California kid and GM262G the signals were not detected.

The signals were detected by Southern hybridization for the genome of the type strain California kid with the probes *mcs4a* and *mcs4b*. The signal was detected of the strain GM262G only with the probe *mcs4a*.

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