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PLASMIDS IN *MYCOPLASMA* SPECIES ISOLATED FROM GOATS AND SHEEP AND THEIR PRELIMINARY TYPING [a]

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ABSTRACT

One-hundred-five (105) clinical isolates of mycoplasma from caprine origin and one isolate from ovine were surveyed for plasmids, which were present in thirty-three (31%) of them. These mycoplasmas originated from 13 herds. Ten of them were symptomatic for mycoplasmal disease (mastitis, polyarthritis, septicemia) and three herds were asymptomatic, i.e., clinically normal. Twenty-eight isolates were *Mycoplasma mycoides* subspecies *mycoides* LC (large colony or caprine biotype), four were *Mycoplasma capricolum* subsp. *capricolum* and one was *Mycoplasma cottewii*. The isolated plasmids were linearized by *Eco*RI, *Eco*RV, *Eco*RI and *Eco*RV or *Bam*HI and *Eco*RV, and were of five sizes (1.1, 1.6, 1.7, 1.8, and 1.9 Kbp). Based on restriction enzyme digestion and size of the linearized supercoiled extrachromosomal DNA, five plasmid types were recovered (p1II, p2III, p2V, p3I, and p4IV). The small size of these DNA elements probably exclude replicative forms of DNA virus, which are equal or larger than 8.0 Kbp.

Key words: *Mycoplasma*, plasmid, goat, endonuclease, electrophoresis

INTRODUCTION

Plasmids in *Mycoplasma* species (class Mollicutes) are unusual or rare, and only a few have been documented. Most extrachromosomal DNA isolated from mollicutes have been from viruses that are specific for mycoplasma, and the majority of the extrachromosomal DNA studies among these mycoplasma have been conducted on members of the genus *Spiroplasma* because of the availability of these nucleic acid elements in these organisms (15). Since the isolation of satellite DNA from *Mycoplasma arthritidis* (12) and *M. hominis* (19) few studies relating to the isolation of plasmids from members of the genus *Mycoplasma* have been reported. Since then, plasmids have been isolated from an unspeciated mycoplasma recovered from a baboon (13), and from a caprine strain of *M. mycoides* subsp. *mycoides* (1,2,8). Thereafter, an unspeciated mycoplasma isolated from a goat was found to contain extrachromosomal DNA of probable plasmid origin (6).

Despite the presence of some reports concerning plasmid isolations from mycoplasma, the number of isolated plasmid types is small and there is little

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information on the function of these DNA elements as compared to bacteria. Additionally, information on transcriptional differences, between bacteria plasmid and the mycoplasma system (14) has incited more research in this area.

In the present study, we report on the occurrence of plasmids in 106 caprine or ovine mycoplasmal isolates (105 caprine, 1 ovine) recovered from 10 diseased and three asymptomatic herds, i. e., clinically normal goats.

MATERIALS AND METHODS

Mycoplasma strains used: One-hundred-five caprine and one ovine (isolate GM630A, Table 1) mycoplasma isolates were examined. The isolates belonged do Dr. Al J. DaMassa Collection, Department of Population Health and Reproduction, University of California, Davis, USA, and only those with retrievable informations were used. They originated from separate farms and included mycoplasma from three asymptomatic and 10 symptomatic herds showing mastitis, polyarthritis, or septicemia either singly or in combination (Table 1). *M. cottewii*, type strain VIS, (7), was obtained from G. S. Cottew, CSIRO, Division of Animal Health, Parkville, Victoria, Australia. With the exception of *M. cottewii,* and isolates from herds "C", GM261B and GM267C, recovered from the external ear canal, and GM1015A (Table 1), all other mycoplasma under study originated from caprine (nine herds) or ovine (one herd) located in the Central Valley of California, USA, which had been involved in outbreaks of mycoplasmosis as reported previously (4,5,6,9). Additionally, *M. mycoides* subsp. *mycoides* strain GM12 (4) was used as a positive control because it contains a plasmid of about 1.85 Kbp (8). Prior to identification each mycoplasma isolate was filter-cloned a minimum of two times through 300 nm filters according to a procedure described elsewhere (18). For purposes of this study, the term *M. mycoides* subsp. *mycoides* will refer only to "large colony or caprine biotypes" and not to bovine or "small colony" forms of the organism.

Mycoplasma strains and identification: Mycoplasmas were identified by a growth-inhibition procedure (3) modified by the use of agar wells rather than discs.

Growth media, culturing, and processing: All isolates were grown for 24 to 48 hours in 50 ml of modified Hayflick liquid medium "B" described elsewhere (10). The cultures were centrifuged for 15 minutes at 20,000 x G. The cells pellets were washed twice in PBS, pH 7.4, resuspended in 5 ml of PBS and stored in 1 ml aliquots at –20°C for subsequent use.

DNA extraction and digestion: The DNA was extracted from mycoplasma cells by an alkaline lysis mini-preparation procedure (17). Aliquots of the sedimented DNA from each extraction were electrophoresed in agarose gel, stained with ethidium bromide, visualized under ultraviolet light, and photographed as previously described (16). Aliquots from samples containing supercoiled extrachromosomal DNA were digested with *Bam*HI, *Eco*RI, and *Eco*RV according to a standard protocol (17) for linearization and size determination (16), for preliminary typing. Following digestion, samples from each reaction were electrophoresed, visualized, and photographed as described above.

Virus assay: The supernatant of five plasmid-positive mycoplasma strains (GM30A, GM261B, GM1013, GM1043, GM630A) were assayed for virus by a standard method (19), and also by a plaque assay method described elsewhere (11).

RESULTS

Mycoplasma strains: The mycoplasma examined in this study were identified either as *M. capricolum* subsp. *capricolum*, *M. mycoides* subsp. *mycoides* LC (large colony or caprine biotype), or as *M. cottewii.*

Data pertaining to the identity of the *Mycoplasma*, the animal species from which it was isolated, the plasmid types that were recovered, and the clinical signs caused by the mycoplasma in the herd in question are presented in Table 1. Of the 106 mycoplasma isolates examined, 31.1% (33/106) contained plasmids. The plasmid-positive isolates were recovered from 13 different herds; 12 from California, USA, and one from Australia. Of the 13 plasmid-positive herds, 10 were symptomatic for mycoplasmal disease (mastitis, polyarthritis, septicemia, singly or in combination), and 3 were asymptomatic; one of the symptomatic herd was of sheep origin (Table 1). Of the 33 plasmid-positive isolates, 85.0% (28/33) were derived from *M. mycoides* subsp. *mycoides*, 12.0% (4/33) from *M. capricolum* subsp. *capricolum*, and 3.0% (1/33) from *M. cottewii*.

Table 1. Characteristics of the mycoplasma isolates and their recovered plasmid types

Herd	Mycoplasma Species	Disease signs	Isolate-total/ herd	Plasmid size (Kbp)	Digestion assay	Plasmid types
A	Mmm	P	1	1.8	*Eco*RI	p1II
B	Mmm	M, P, S	18	1.8	*Eco*RI	p1II
C	Mmm	None[a]	1	1.8		p1II
	Mcc	None	2	1.1	*Eco*RV	*Eco*RI p2V
D	Mmm	P	1	1.8	*Eco*RI	p1II
E	Mmm	P	1	1.8	*Eco*RI	p1II
F	Mmm	P	1	1.7	*Eco*RV	p2III
G	Mmm	P	1	1.9	*Eco*RI/V	p3I
H	Mmm	P	2	1.8	*Eco*RI	p1II
I	Mmm	P	1	1.8	*Eco*RI	p1II
J	Mmm	M	1	1.7	*Eco*RV	p2III
K	Mcc[b]	P	1	1.1	*Eco*RV	p2V
L	Mcc	None	1	1.8	*Eco*RI	p1II
M	Mci	None[a]	1	1.6	*Bam*/*Eco*	p4IV

Mmm = *Mycoplasma mycoides* subsp. *mycoides*, *M* = mastitis, P = polyarthritis, S = septicemia, a = mycoplasma recovered from the external ear canal, Mcc = *Mycoplasma capricolum* subsp. *capricolum,* b = isolate GM630A from sheep, *Eco*RI/V = *Eco*RI and *Eco*RV, Mci = *Mycoplasma cottewii*, and *Bam*/*Eco* = *Bam*HI and *Eco*RV.

Mycoplasma virus and plasmids: Virus was not recovered from culture supernatant or by the plaque assay procedure from the five mycolasma isolates that were studied. The results and/or characteristics pertaining to the recovered plasmids are shown in Table 1, Fig. 1 and Fig. 2. Based on restriction enzyme digestion, the plasmids were arbitrarily assigned to groups 1 to 4 depending on whether they were linearized, respectively, by *Eco*RI, by *Eco*RV, by *Eco*RI and *Eco*RV, or by *Bam*HI and *Eco*RV, (Fig. 1). They were further assigned to subgroups I to V according to their approximate size (Kbp) of 1.9 (one digested by *Eco*RI and *Eco*RV), 1.8 (all digested by *Eco*RI), 1.7 (two digested by *Eco*RV), 1.6 (one digested by *Bam*HI and *Eco*RV) and, 1.1 (three digested by *Eco*RV) (Table 1, and Fig.1), respectively. By combining the enzyme digestion reaction and the size of the linearized form, five different types of plasmid were found, namely p1II in 25 isolates of *M. mycoides* subsp. *mycoides* and in one isolate of *M. capricolum* subsp. *capricolum*; p2III found in two isolates of *M. mycoides* subsp. *mycoides*; p2V found in three isolates of *M. capricolum* subsp. *capricolum*; p3I found in one isolate of *M. mycoides* subsp. *mycoides* and p4IV found in *M. cottewii* strain GM612 (Table 1). Only one plasmid type was detected in any single isolate, but a particular mycoplasma species was found to carry more than a single plasmid type. For example, different isolates of *M. mycoides* subsp. *mycoides* carried plasmid types p1II, p2III and p3I (Table 1). All the 18 *M. mycoides* subsp. *mycoides* strains from herd B, which were recovered during one outbreak of mastitis and polyarthritis, contained the same type of plasmid (Table 1, and Fig. 2).

DISCUSSION

Although all the plasmid-positive mycoplasma isolates were not routinely assayed for the presence of virus, the sizes of the recovered extrachromosomal DNAs were were small enough (1.1 to 1.9 Kbp) to exclude replicative forms (RF) of viral DNA. The size of the reported RF DNAs from mollicutes is equal to or over than 8.0 Kbp (15).

The rate of occurrence of plasmids among the field strains of caprine mycoplasmas in this study was relatively high, in contrast with a previously reported plasmid survey on mycoplasmas from various animal hosts (13). In the United States, severe large-scale outbreaks of caprine mycoplasmosis are on record (4,5,6,9), particularly from California, which was the origin of all except one of the isolates used in this study. The extensive use of antimicrobial drugs in

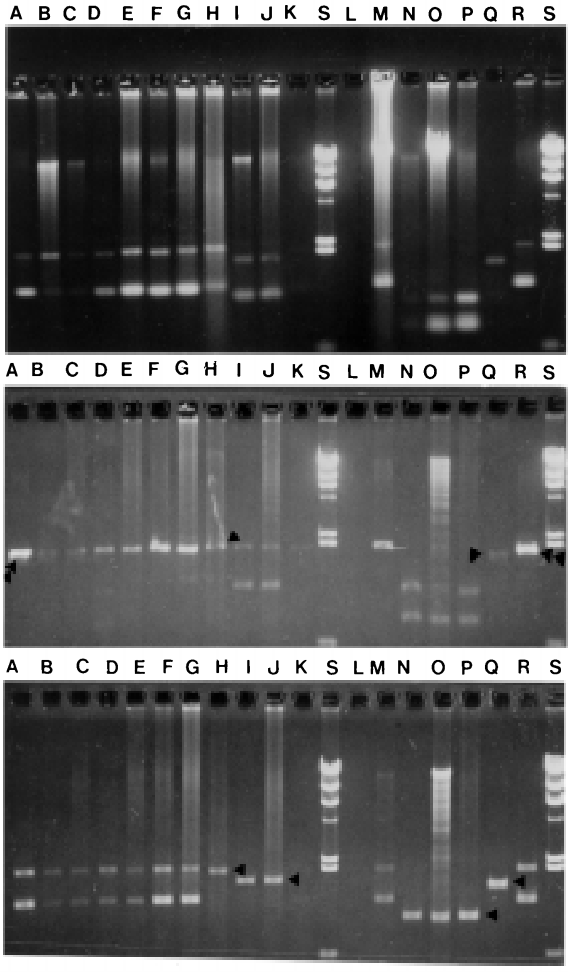

Figure 1. Agarose gel electrophoresis of plasmids from mycoplasmas recovered from 16 mycoplasma isolates under study. Top figure represents supercoiled (uncut) plasmids. Middle, represents *Eco*RI digested plasmids. Bottom, represents *Eco*RV digested plasmids. A and R = plasmid from GM12 (control), B = GM1031B, C = GM1031G, D = GM728A, E = GM975, F = GM262E, G = GM3, H =GM1019, I = GM1013, J = GM1053, K = GM1043, L = blank, M = GM1015A, N = GM630A, O = GM267C, P = GM261B, and Q = GM612. Lanes S represent molecular-weight standard (*Hin*dIII-cleaved lambda phage DNA). Single-arrows indicate the size in base pair of the linearized plasmids. Double-arrows indicate the size (1.85 Kbp) of the linearized pKMK1 plasmid recovered from *M. mycoides* subsp. *mycoides* strain GM12 (Dybvig and Khaled, 1990).

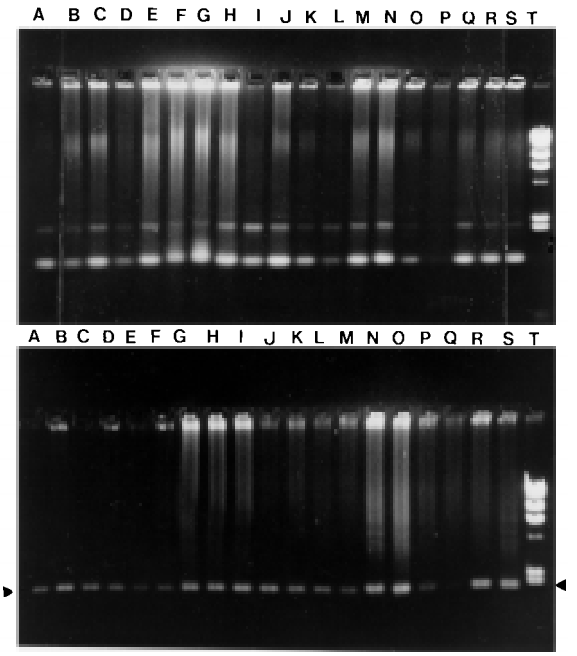

Figure 2. Agarose gel electrophoresis of plasmids from mycoplasmas recovered from all mycoplasma isolates of herd B. Top figure represents supercoiled (uncut) plasmids. Bottom, represents *Eco*RI digested plasmids. A = plasmid from GM12 (1.85 Kbp, control), B = GM32G, C = GM32D, D = GM28F, E = GM32B, F = GM32H, G = GM32A, H = GM28H, I = GM32C, J = GM32E, K = GM28D, L = GM30A, M = GM32J, N = GM28G, O = GM30B, P = GM32K, Q = GM30G, R = GM30L, and S = GM28C. Lane T represents molecular-weight standard (*Hin*dIII-cleaved lambda phage DNA). The arrows indicate the migration size in Kbp of the linearized plasmids.

these herds may have contributed to the high occurrence of plasmid in the mycoplasma strains isolated in this study. Most of the plasmids were recovered from *M. mycoides* subsp. *mycoides* isolates, but *M. capricolum* subsp. *capricolum* and *M. cottewii* were also found to contain plasmids. Excluding one plasmid from a single symptomatic sheep herd studied, most of them (26/33) originated from mycoplasma isolates from disease outbreaks in caprines. Plasmids of different types were found in different isolates of both *M. mycoides* subsp. *mycoides* and *M. capricolum* subsp. *capricolum*.

Based on size and herd of origin the p1II plasmid is probably of the same type as the pKMK1 described previously (8). Plasmids of about 1.7 Kbp have been reported previously (1), but there seems to exist no previous report of a mycoplasma plasmid as small as 1.1 Kbp. The finding of new types of plasmids, more importantly the smaller ones, because they can easily be sequenced and engineered, may add to gene expression studies in mycoplasmas as bacterial plasmids are not fully compatible with the transcription system of the former organism as evidenced previously (14). It is hoped that some of the findings reported in this study will add further to the understanding of the role of plasmids within mycoplasma cells, as well as in mycoplasma infection.

RESUMO

Plasmídios em espécies de *Mycoplasma* isoladas de caprinos e de ovinos e sua tipagem preliminar

Um total de 105 amostras clínicas de micoplasma, originárias de caprinos, e uma de ovino foram investigadas quanto a presença de plasmídios, que foram observados em trinta e três (31%) delas. Esses micoplasmas provieram de 13 rebanhos. Dez desses rebanhos eram sintomáticos para micoplasmose (mastite, poliartrite, septicemia) e três eram assintomáticos, i.é., clinicamente sadios. Vinte e oito amostras eram *Mycoplasma mycoides* subespécie *mycoides* LC ("large colony" ou biotipo caprino), quatro eram *M. capricolum* subsp. *capricolum* e uma era *M. cottewii*. Os plasmídios foram linearizados com endonucleases *Eco*RI, *Eco*RV, *Eco*RI e *Eco*RV ou *Bam*HI e *Eco*RV e apresentaram cinco tamanhos (1,1; 1,6; 1,7; 1,8 e 1,9 Kbp). Com base na digestão pela enzima de restrição e o tamanho dos DNA extracromossomais, cinco tipos de plasmídio foram encontrados (p1II, p2III, p2V, p3I e p4IV). O reduzido tamanho desses DNA circulares muito provavelmente exclui formas replicativas de vírus DNA, cujo tamanho é igual ou maior que 8,0 Kbp.

Palavras-chave: *Mycoplasma*, plasmídio, caprino, endonuclease, eletroforese

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