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A Species-Specific DNA Probe for the Detection of *Mycoplasma gallisepticum*

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An 800-base-pair DNA fragment from a partial genomic library of *Mycoplasma gallisepticum* was selected and used as a probe for the selective detection of this avian pathogen. The specificity and sensitivity of this probe were demonstrated by using dot blot and Southern hybridizations.

Detection and identification of mycoplasmas are based on their cultivation from infected tissues and on biochemical and serological assays (16). Cultivation techniques are laborious and expensive and need sterile conditions and time, while biochemical assays are relatively nonspecific (13). Serological methods are important tools in the classification of mycoplasma strains but they have limited value in the diagnosis of mycoplasma infection of animals (16). However, molecular cloning of mycoplasmal nucleic acids and subsequent selection of specific gene probes have led to a new approach to the detection of mycoplasmas. The first probe containing cloned rRNA of *Mycoplasma capricolum* and *M. hyorhinis* could detect and differentiate mycoplasmas in tissue cultures by Southern hybridization but could not identify the contaminating mycoplasma species by the simple and rapid dot blot hybridization (7, 12, 15). The detection of *M. hyopneumoniae* from lung washings of experimentally infected pigs was demonstrated by using a cloned DNA fragment of this species as a probe in Southern hybridization (3). Recently, Taylor et al. (15) reported the use of two cloned genomic DNA fragments from *M. hyorhinis* to specifically identify this species by both Southern and dot hybridizations. In the present study, a partial genomic library of the avian pathogen *M. gallisepticum* was constructed and an 800-base-pair (bp) cloned DNA fragment was selected for the differential detection of this species.

Mycoplasmal, acholeplasmal, and bacterial DNAs were prepared by the method of Chan and Ross (3), and eucaryotic DNAs were purified as described by Gross-Bellard et al. (8). Total DNA of *M. gallisepticum* S6 Holland was digested by restriction enzyme *Sau*3A, and the generated DNA fragments were separated on a Sepharose CL-4B column (11). Mycoplasmal DNA fragments longer than approximately 300 bp were ligated with pBR322 digested by *Bam*HI (11). Transformation of *Escherichia coli* C600 cells was done by the calcium chloride method (10). Ampicillin-resistant, tetracycline-sensitive colonies were selected and screened by the procedure of Birnboim and Doly (1). The lengths of the inserted mycoplasmal DNA fragments were estimated by gel electrophoresis of recombinant plasmids linearized by *Pst*I (11). Inserts from six recombinant plasmids were isolated by electroelution (17), labeled by the random priming method (5), and used as probes in dot blot hybridizations (2) against various mycoplasmal and bacterial DNAs. One probe, an 800-bp cloned mycoplasmal DNA fragment, derived from recombinant plasmid pMg5, was selected and used throughout this study.

This probe recognized the homologous DNA sequences of original *M. gallisepticum* S6 Holland and the referent *M. gallisepticum* X95 but failed to hybridize to DNAs purified from nine other avian mycoplasma species, i.e., *M. cloacale* 1221; *Mycoplasma* sp. strains 1219, 1220, and 132/1a; *M. pullorum* CKK; *M. gallinaceum* DD; *M. gallopavonis* WR1; *M. gallinarum* PG16; *M. meleagridis* 17529; from *Acholeplasma axanthum* S743 and *Acholeplasma laidlawii* PG8; and from six bacterial strains, i.e., *E. coli*, *Staphylococcus epidermidis*, *Corynebacterium* sp. strain 187, and two isolates of *Salmonella typhimurium* and *Salmonella gallinarum* frequently present in clinical materials (Fig. 1 and Table 1). It is essential that a probe used for diagnostic purposes not hybridize to eucaryotic DNA. This was investigated by Southern hybridization (14). No positive reaction with the *Sau*3AI-digested DNA fragments of different cell cultures (Chinese hamster ovary [CHO], mouse teratocarcinoma [F9], human [HeLa]) and tissues (chicken erythrocytes,

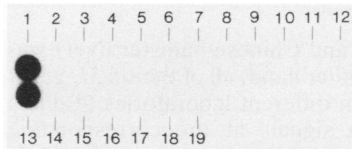

FIG. 1. Dot hybridization of the 800-bp cloned DNA fragment of *M. gallisepticum* to various mycoplasmal and bacterial DNAs. Genomic DNAs (1 μg) from the species tested were bound to nitrocellulose filters by the NaI method of Bresser and Gillespie (2). Hybridization was performed at 37°C for 16 h in a mixture of 50% formamide, 5× SSC (1× SSC is 0.15 M NaCl plus 0.015 M sodium citrate), 0.1% sodium dodecyl sulfate, and the denatured 800-bp *M. gallisepticum* probe (specific activity, 3×10^8 cpm/μg). The filter was washed as previously described (2) and autoradiographed for 30 min. Spots: 1, *M. gallisepticum* S6 Holland; 2, *M. cloacale* 1221; 3, *Mycoplasma* sp. strain 1219; 4, *Mycoplasma* sp. strain 1220; 5, *Mycoplasma* sp. strain 132/1a; 6, *M. pullorum* CKK; 7, *M. gallinaceum* DD; 8, *M. gallopavonis* WR1; 9, *M. gallinarum* PG16; 10, *M. meleagridis* 17529; 11, *A. axanthum* S743; 12, *A. laidlawii* PG8; 13, *M. gallisepticum* X95; 14, *E. coli*; 15, *Staphylococcus epidermidis*; 16, *Corynebacterium* sp. strain 187; 17, *Salmonella typhimurium* (chicken); 18, *Salmonella gallinarum*; 19, *Salmonella typhimurium* (avian).

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TABLE 1. Mycoplasmal and bacterial strains used

Strain	Origin	Source[a]
Mollicutes		
M. gallisepticum		
X95[b]	Chicken trachea	E. A. Freundt
MK7[b]	Chicken airsac	T. Shimizu
MS16[b]	Chicken embryo, dead in shell	T. Shimizu
S6 Holland[b]	Turkey brain	D. Seinstra
S6 USA[b]	Turkey brain	H. E. Adler
S6 Bench[b]	Turkey brain	G. A. Cullen
M. meleagridis 17529[c]	Turkey infraorbital sinus	E. A. Freundt
M. gallinarum PG16[c]	Chicken trachea	H. Erno
M. pullorum CKK[b]	Chicken trachea	D. G. Edward
M. gallinaceum DD[b]	Chicken trachea	D. G. Edward
M. gallopavonis WR1[b]	Turkey airsac	F. T. W. Jordan
M. cloacale 1221[c]	Goose phallus lymph	VMRI
Mycoplasma sp. strain		
1219[c]	Goose phallus lymph	VMRI
1220[b]	Goose phallus lymph	VMRI
132/1a[b]	Goose airsac	VMRI
A. laidlawii PG8[b]	Sewage	E. A. Freundt
A. axanthum S743[b]	Murine tissue cell line	J. G. Tully
Bacterial strains		
E. coli[d]	Turkey	VMRI
Staphylococcus epidermidis[d]	Chicken	VMRI
Corynebacterium sp. strain 187[d]	Chicken	VMRI
Salmonella typhimurium[d]	Chicken	VMRI
Salmonella gallinarum[d]	Chicken	VMRI
Salmonella typhimurium[d]	Avian	VMRI

[a] VMRI, Veterinary Medical Research Institute, Hungarian Academy of Sciences.
[b] Grown in medium B (4) supplemented with 1% glucose.
[c] Grown in medium B (4) supplemented with 1% arginine.
[d] Grown in LB broth (11).

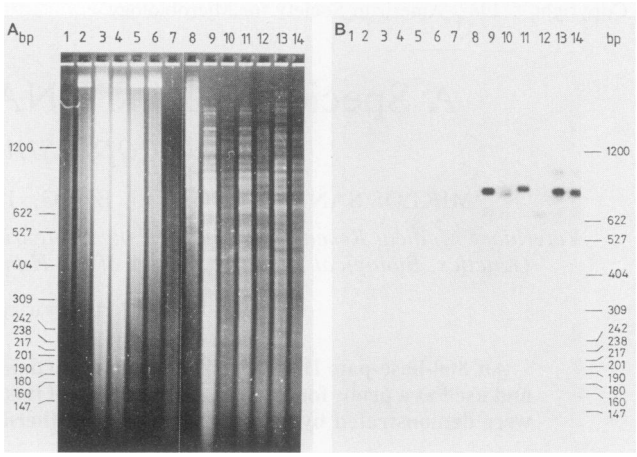

FIG. 2. (A) Agarose gel electrophoresis of *Sau*3AI-restricted genomic DNA isolated from different eucaryotic cells, *M. gallisepticum* strains, *M. gallinarum*, and *Corynebacterium* sp. strain 187. Digests were separated on a 2% agarose gel. (B) Posthybridization autoradiograph. DNA fragments from the gel in panel A were transferred to nitrocellulose filters by the procedure of Southern (14) and hybridized with the the 800-bp *M. gallisepticum* probe (specific activity, 5×10^8 cpm/μg) in a solution of 6× SSC, 0.01 M ethylenediaminetetraacetic acid, 5× Denhardt solution (1× Denhardt solution is 0.02% Ficoll [Pharmacia Fine Chemicals], 0.02% polyvinylpyrrolidone, and 0.02% bovine serum albumin), 0.5% sodium dodecyl sulfate, and 100 μg of denatured salmon sperm DNA per ml at 68°C for 14 h as described previously (11). Autoradiography was performed for 3.5 h. Lanes (*Sau*3AI-digested DNA from): 1, Chinese hamster liver; 2, mouse liver; 3, chicken erythrocytes; 4, Chinese hamster ovary cell line (CHO); 5, mouse teratocarcinoma cell line (F9); 6, human cell line (HeLa); 7, *Corynebacterium* sp. strain 187; 8, *M. gallinarum* PG16; 9, *M. gallisepticum* S6 Holland; 10, *M. gallisepticum* S6 USA; 11, *M. gallisepticum* S6 Bench; 12, *M. gallisepticum* X95; 13, *M. gallisepticum* MK7; 14, *M. gallisepticum* MS16.

mouse liver, and Chinese hamster liver) was obtained (Fig. 2B). On the other hand, all of the six *M. gallisepticum* strains received from different laboratories (Table 1) gave positive hybridization signals at the correspondingly sized DNA fragment. In addition, as was expected from our earlier observation (M. Santha, K. Lukacs, K. Burg, S. Bernath, I. Rasko, and L. Stipkovits, submitted for publication), polymorphism in the hybridization patterns of the six strains was found (Fig. 2B, lanes 9 to 14). The sensitivity of this probe was determined by dot hybridization. Purified DNA of *M. gallisepticum* S6 Holland (0.5 ng) was detected after an autoradiography of 6 h (Fig. 3). This amount of DNA is roughly equivalent to the DNA content of 5×10^4 mycoplasma cells calculated by the method of Razin et al. (13).

The high specificity of a cloned genomic DNA fragment of *M. gallisepticum* was demonstrated in the present work. Obviously, further investigations are necessary to increase the sensitivity of the assay, so that *M. gallisepticum* can be directly identified from clinical materials. Replacement of the ^{32}P-labeled probe with a harmless biotinylated one (6, 9) would make possible the extended use of this probe in routine laboratories for the detection of *M. gallisepticum* strains.

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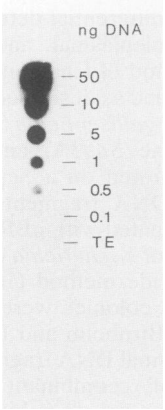

FIG. 3. Dot hybridization of the 800-bp cloned DNA probe (specific activity, 1.5×10^9 cpm/μg) to various dilutions of purified DNA of *M. gallisepticum* S6 Holland. Application of DNA to nitrocellulose filters and dot hybridization were done as described in the legend to Fig. 1. Autoradiography was performed for 6 h. TE, TE buffer (10 mM Tris hydrochloride [pH 8.0], 1 mM EDTA).

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