Electrophoretic Patterns of Membrane Proteins of *Mycoplasma*

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Cell membranes of *Mycoplasma* were isolated either by osmotic lysis or by ultrasonic disruption of the organisms. The membranes were dissolved in phenol-acetic acid-water (2:1:0.5, w/v/v), and membrane proteins were separated electrophoretically in polyacrylamide gels containing 5 M urea and 35% (v/v) acetic acid. The electrophoretic patterns of membrane proteins were highly specific for the different *Mycoplasma* strains examined. The use of this method to prove the identity or dissimilarity of *Mycoplasma* strains is suggested.

The *Mycoplasma* cell is bounded by a single membrane composed of 50 to 60% protein and 30 to 40% lipid (14). The major part of the protein of *Mycoplasma laidlawii* membranes could be separated from membrane lipid after disaggregation of the membranes by detergents. The purified protein fraction was highly hydrophobic, resembling the structural protein of mitochondrial membranes (A. W. Rodwell et al., *to be published*). When dissolved in sodium dodecyl sulfate, this protein fraction migrated as a single heavy band in polyacrylamide gels prepared according to Davis (2). However, when the protein was dissolved according to Takayama et al. (19) in phenol-acetic acid-water (2:1:0.5, w/v/v) and was run in polyacrylamide gels containing 5 M urea and 35% acetic acid, more than 10 protein bands were resolved (A. W. Rodwell et al., *to be published*). The acetic acid and urea served to transform the hydrophobic membrane proteins to their monomeric form and prevented the reaggregation of the monomers during migration in the gels (19). Further study has shown that isolated *M. laidlawii* membranes could also be dissolved in phenol-acetic acid-water (2:1:0.5, w/v/v). The dissolved membrane proteins exhibited a highly reproducible electrophoretic pattern when tested in Takayama's system. The pattern was the same for membranes isolated from different batches of cells. This led us to compare the electrophoretic patterns of membrane proteins of different *Mycoplasma* strains, and since the protein component of the membrane is genetically determined, its electrophoretic pattern should be characteristic for each *Mycoplasma* species. The results presented show that the electrophoretic patterns of membrane

proteins can serve as useful "fingerprints" for the identification of *Mycoplasma*.

MATERIALS AND METHODS

Organisms and growth conditions. The designation and source of the *Mycoplasma* strains examined are given in Table 1. The organisms were grown statically in 500 ml of a modified Edward medium (13) containing 2% (v/v) PPLO Serum Fraction (Difco). The organisms were harvested after 24 to 48 hr of incubation at 37 C by centrifugation at 13,000 × *g* for 10 min. The sedimented cells were washed twice in 0.25 M NaCl.

Isolation of cell membranes. Cell membranes were isolated either by osmotic lysis of the organisms (13) or by disruption of the cells by ultrasonic treatment. For osmotic lysis, the washed sedimented cells were resuspended in 100 to 200 ml of deionized water and were incubated at 37 C for 15 min. The treated suspensions were centrifuged at 8,000 × *g* for 5 min to remove clumps of unbroken cells. The supernatant fraction was centrifuged for 30 min at 34,000 × *g* to collect membranes. Ultrasonic treatment was carried out in an MSE ultrasonic disintegrator (60 w). A 7-ml amount of a heavy suspension of the washed organisms in deionized water was treated at 1.5 amp for 3 min. The suspension was then centrifuged at 34,000 × *g* for 30 min to collect membranes. The membranes were washed 10 times alternatively with deionized water and 0.05 M NaCl in 0.01 M phosphate buffer (pH 7.5). The washed membranes were resuspended in deionized water and kept at −20 C until used. The amount of protein in membrane suspension was determined according to Lowry et al. (11). Nucleic acids were extracted according to Schneider (18). Ribonucleic acid (RNA) was determined by the method of Drury (3) and deoxyribonucleic acid (DNA), according to Burton (1).

Polyacrylamide gel electrophoresis. The gels contained 7.5% (w/v) acrylamide, 35% (v/v) acetic acid, and 5 M urea in 6- by 100-mm glass tubes (19). For the

TABLE 1. Source of Mycoplasma strains

Type or species	Strain	Recovered from	Obtained from
M. gallisepticum	PG31	Chicken	D. G. ff. Edward[a]
	S6	Chicken	H. E. Adler[b]
	A5969	Chicken	M. E. Tourtellotte[c]
M. gallinarum	J	Chicken	P. F. Smith[d]
M. meleagridis	N	Turkey	R. Yamamoto[b]
Mycoplasma sp.	R	Chicken	R. Bernstein-Ziv[e]
M. mycoides var. mycoides	PG1	Cattle	D. G. ff. Edward[a]
	V5	Cattle	A. W. Rodwell[f]
M. mycoides var. capri	PG3	Goat	D. G. ff. Edward[a]
Mycoplasma sp.	Y	Goat	A. W. Rodwell[f]
Mycoplasma sp.	14	Goat	M. E. Tourtellotte[c]
Mycoplasma sp.	pp. goat	Goat	E. Klieneberger-Nobel[g]
M. laidlawii	A	Sewage	D. G. ff. Edward[a]
	OR	Human oral cavity	Isolated in our laboratory
	B	Sewage	D. G. ff.[Edward[a]
	B	Sewage	M. E. Tourtellotte[c]

[a] Wellcome Research Laboratories, Beckenham, Kent, England.
[b] School of Veterinary Medicine, Univ. of California, Davis.
[c] University of Connecticut, Storrs.
[d] University of South Dakota, Vermillion.
[e] Faculty of Agriculture, The Hebrew University, Rehovot, Israel.
[f] C.S.I.R.O. Animal Health Research Laboratory, Parkville, Victoria, Australia.
[g] The Lister Institute of Preventive Medicine, London, S.W.1.

preparation of the gels, 3 ml of a stock solution containing 0.3 g of acrylamide, 0.6 g of urea, and 0.008 g of N,N'-methylenbisacrylamide in 47% (v/v) acetic acid was mixed with 1 ml of a fresh solution containing 0.6 g of urea, 0.015 g of ammonium persulfate, and 0.02 ml of N,N,N',N'-tetramethylethylenediamine. Each glass tube was filled with 1.8 ml of the mixture and was overlayered with 75% (v/v) acetic acid. Polymerization of the acrylamide was carried out at 37 C for 45 min. The membranes were dissolved by adding 2 volumes of phenol-acetic acid-water (2:1:0.5, w/v/v) to 1 volume of membrane suspension. A 20- to 100-µliter amount of the sample to be analyzed (100 to 300 µg of protein) was mixed with 50 µliters of a 40% (w/v) sucrose solution in 35% (v/v) acetic acid and was put on top of the gel. Then, 0.5 ml of 75% (v/v) acetic acid was carefully layered over the sample-sucrose mixture, and the tube was filled up with a solution of 10% (v/v) acetic acid. Both upper and lower reservoirs of the electrophoresis apparatus were filled with 10% (v/v) acetic acid. The lower electrode served as a cathode, and electrophoresis was carried out at room temperature for 2 hr at a constant current of 5 ma per tube. The gels were stained with 1% (w/v) Amido Black 10B in 7% (v/v) acetic acid for 1 hr and were then rinsed well with tap water. For destaining, the gels were immersed overnight in 7% (v/v) acetic acid. The residual background stain was removed electrolytically in 7% (v/v) acetic acid by use of a direct current of 200 ma for 2 hr.

RESULTS

All the batches of membranes prepared from the different Mycoplasma strains were tested for

their RNA and DNA content. The membrane preparations were found to contain only 1 to 2% by weight of nucleic acids, indicating a low degree of cytoplasmic contamination. In several cases, the "soluble" or cytoplasmic fraction of the cells, obtained during membrane isolation, was analyzed electrophoretically. The electrophoretic pattern obtained was completely different from that of the membrane fraction, again indicating the relative freedom of the membrane fraction from soluble proteins. The electrophoretic patterns of all strains examined were highly reproducible and did not vary significantly in different batches of membranes prepared from the same organism.

The electrophoretic patterns of the avian mycoplasmas are presented in Fig. 1 and Fig. 2, and schematically in Fig. 3. Strains S6 and PG31 of M. gallisepticum possess identical electrophoretic patterns, whereas strain A5969 is distinguished only by one missing band (Fig. 2 and 3). The other three avian strains showed characteristic electrophoretic patterns differing from that of M. gallisepticum and from each other.

The electrophoretic patterns of the bovine and caprine strains were specific for each strain. Thus, the two strains of M. mycoides var. mycoides markedly differed from each other, as did the four goat strains examined (Fig. 4 and 5).

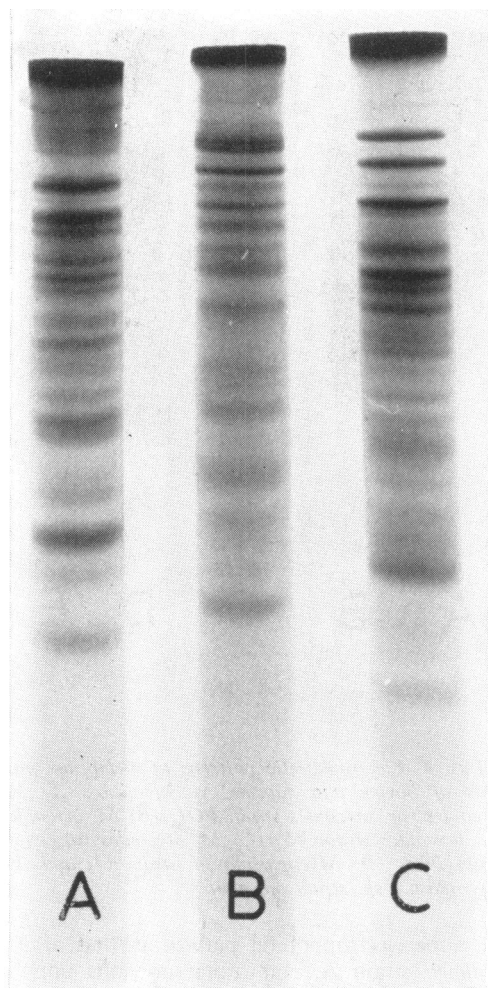The saprophytic M. laidlawii strains showed two different electrophoretic patterns, one shared

FIG. 2. *Electrophoretic patterns of membrane proteins of avian Mycoplasma. (A) M. gallisepticum strain S6; (B) M. gallisepticum strain A5969; and (C) Mycoplasma sp. strain R.*

FIG. 1. *Electrophoretic patterns of membrane proteins of avian Mycoplasma. (A) M. meleagridis strain N; (B) M. gallisepticum strain A5969; and (C) M. gallinarum strain J.*

by *M. laidlawii* strain A and the oral strain, and the other by the two *M. laidlawii* B strains (Fig. 6).

DISCUSSION

Mycoplasma strains are currently differentiated by antigenic variations, biochemical properties, host specificity, and tissue localization of the organisms. The recent application of nucleic acid homology techniques has provided the most direct approach to the assessment of relatedness among mycoplasmas (16, 17). However, these techniques are still too complex to be used for large-scale comparative studies. Since there is little doubt that
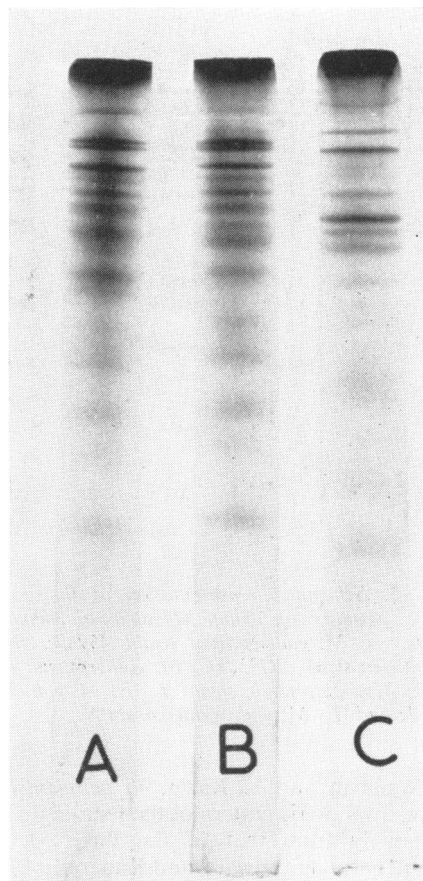
the synthesis of membrane proteins is genetically directed (8), it seemed reasonable to assume that the electrophoretic pattern of membrane proteins should reflect genetic identity or nonidentity of microorganisms. Our results appear to confirm this by showing the electrophoretic patterns of the various species to be specific and correlated with serological data.

Of the avian mycoplasmas investigated, three strains, PG31, S6, and A5969, showed an identical, or almost identical, electrophoretic pattern. This is in accordance with previous serological studies which placed these strains in a single serological group comprising the species *M. gallisepticum* (4, 5, 7). The R strain, isolated from chicken suffering from chronic respiratory disease in Israel, appears to be different from the *M. gallisepticum* strains, both in its electrophoretic pattern and in its serological behavior
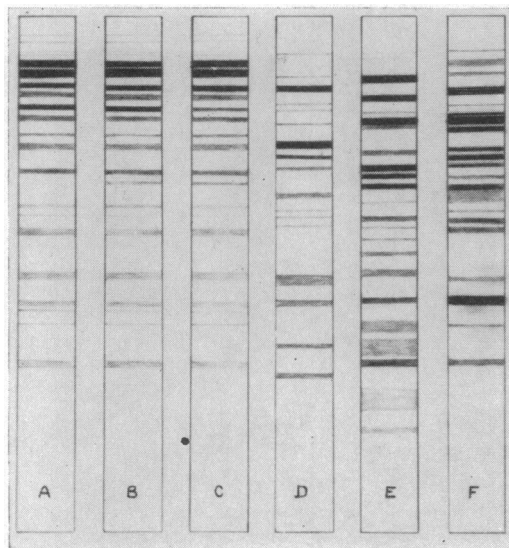
FIG. 3. Schematic representation of the electrophoretic patterns of avian Mycoplasma membrane proteins. (A) M. gallisepticum strain A5969; (B) M. gallisepticum strain PG31; (C) M. gallisepticum strain S6; (D) Mycoplasma sp. strain R; (E) M. gallinarum strain J; and (F) M. meleagridis strain N.

FIG. 4. Electrophoretic patterns of membrane proteins of bovine and caprine Mycoplasma. (A) M. mycoides var. mycoides strain PG1; (B) M. mycoides var. mycoides strain V5; (C) M. mycoides var. capri strain PG3; (D) Mycoplasma sp. strain 14; and (E) Mycoplasma sp. strain pp. goat.

(M. Argaman and S. Razin, to be published). The electrophoretic pattern of the J strain differed considerably from that of the three M. gallisepticum strains. Edward and Kanarek (4) and Kelton and Van Roekel (7), unable to find any serological relationship between this strain and M. gallisepticum, suggested that it be classified as M. gallinarum. The characteristic electrophoretic pattern of the turkey pathogen M. meleagridis lends support to its classification as a separate species (20).

All the bovine and caprine mycoplasmas examined showed different electrophoretic patterns. The type species M. mycoides var. mycoides (PG1) appears to be different from the Australian strain V5. Likewise, M. mycoides var. capri (PG3) differs from the other goat strains. The antigenic differences among the goat mycoplasmas and their confused taxonomical position have been noticed before (10; R. M. Lemcke, personal communication; M. Argaman and S. Razin, to be published).

Both M. laidlawii strains designated B exhibited an identical electrophoretic pattern, despite their cultural differences. The strain obtained from M. E. Tourtellotte can grow in tryptose broth without PPLO Serum Fraction (15), whereas the strain obtained from D. G. ff. Edward cannot. The oral strain of M. laidlawii was found to have
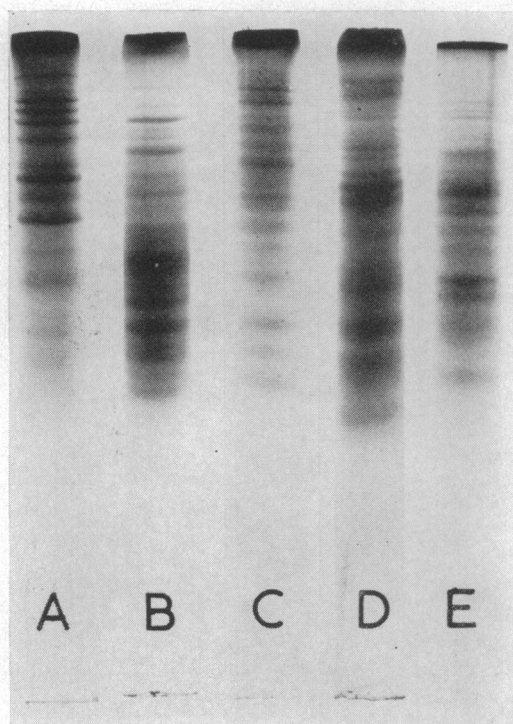
the same electrophoretic pattern as that of M. laidlawii strain A, confirming previous unpublished results of M. Argaman indicating their close serological relationship. The electrophoretic pattern of the A strains differed from that of the B strains, corroborating the original separation based on their antigenic dissimilarity (9).

Fowler et al. (6) first suggested use of electrophoretic patterns of Mycoplasma proteins as a means for characterizing Mycoplasma strains. By starch-gel electrophoresis of the soluble portion of sonically treated organisms, these authors demonstrated that each strain studied had a characteristic protein pattern. The disadvantage of using the "soluble" fraction obtained after sonic disruption of the organisms is that it contains a variable amount of membrane protein (12). The use of purified membranes in our study assured the analysis of a more homogeneous fraction of cell protein. In addition, polyacrylamide-gel electrophoresis is much superior for the separation of proteins to starch-gel electrophoresis.
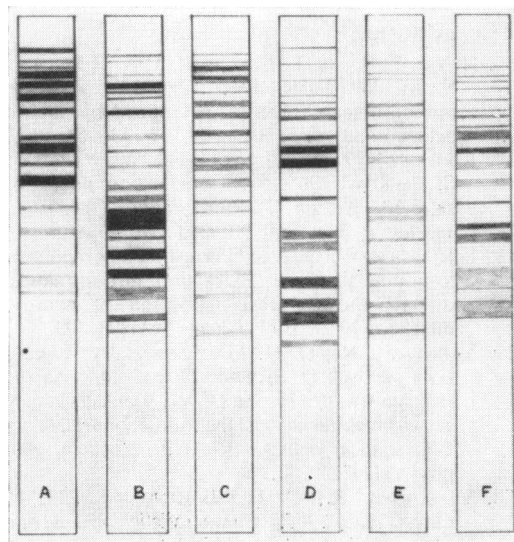
FIG. 5. *Schematic representation of the electrophoretic patterns of bovine and caprine Mycoplasma membrane proteins. (A) M. mycoides var. mycoides strain PG1; (B) M. mycoides var. mycoides strain V5; (C) M. mycoides var. capri strain PG3; (D) Mycoplasma sp. strain 14; (E) Mycoplasma sp. strain Y; and (F) Mycoplasma sp. strain pp. goat.*

Acetic acid was essential in our electrophoretic system to solubilize and separate membrane proteins, whereas urea improved the separation. The high concentration of urea in the gels did not cause any new protein bands to appear, as the decrease or the complete omission of urea from the gels did not change the electrophoretic pattern.

Preliminary experiments carried out in our laboratory show that washed cells of the various *Mycoplasma* strains also exhibit characteristic electrophoretic patterns. The use of washed cells instead of isolated membranes will reduce the amount of organisms required and will simplify the procedure considerably. Identification of *Mycoplasma* by the electrophoretic method is rapid, requires only the type species or strain for comparison, and eliminates the need for expensive antisera.
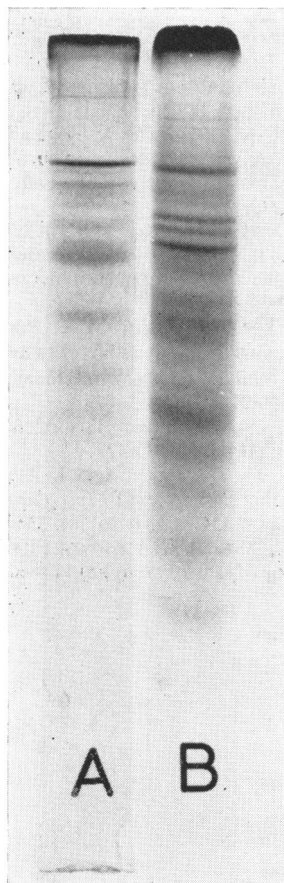
FIG. 6. *Electrophoretic patterns of membrane proteins of Mycoplasma laidlawii. (A) M. laidlawii strain B; (B) M. laidlawii strain A.*

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