Electrophoretic Patterns of Strains of *Mycoplasma pulmonis*

By KATHRYN A. FORSHAW*

Department of Pathology, Ruchill Hospital, Glasgow, G20 9NB

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SUMMARY

Strains of *Mycoplasma pulmonis*, examined by polyacrylamide-gel electrophoresis of proteins extracted in a phenol-acetic acid-water solvent, gave patterns with reproducible differences. Some strains could be placed into groups, but a number could not be associated with any group. In all but one case a 'basic *M. pulmonis* pattern' was easily recognizable. This exception suggests that occasionally one might fail to recognize a strain of *M. pulmonis* by relying solely on the electrophoretic technique.

INTRODUCTION

Fowler, Coble, Kramer & Brown (1963) suggested that the electrophoretic separation of proteins of mycoplasmas be used as a new method of identifying members of this species. Their observations were extended by Rottem & Razin (1967). The patterns obtained by these workers were distinct for the species of mycoplasmas examined. Sometimes there were differences between the electrophoretic patterns of strains within a species; for example, *Mycoplasma pulmonis* (Razin & Rottem, 1967; Razin, 1968).

In the present investigation 19 strains of *Mycoplasma pulmonis* were examined by polyacrylamide-gel electrophoresis to find out to what extent the electrophoretic patterns of their proteins differed and to see if a typing scheme could be formed on the basis of these patterns.

METHODS

Organisms. The strains of mycoplasmas and the sources from which they were obtained are given in Table 1. The strains included reference strains and recent isolates. Preliminary identification of recently isolated strains was made by the growth inhibition test (Clyde, 1964) employing at least two antisera to *Mycoplasma pulmonis* plus antiserum to the other rodent mycoplasmas *M. neurolyticum* and *M. arthritidis*. Strains which were inhibited were regarded as members of the species *M. pulmonis*.

Clones. All strains were cloned at least three times. Where an original culture showed several colonial forms, representative colonies were cloned six times.

Media and growth conditions. The media used for the cultivation of the mycoplasmas were modified from that described by Chanock *et al.* (1962). Thallous acetate was omitted and ampicillin at a final concentration of 0.1 % was used in place of penicillin (Fallon, 1969). Medium which contained 1 % (w/v) glucose and 0.002 % phenol red was inoculated from a logarithmic phase culture and incubated aerobically at 37 °C until a colour change in the medium showed a drop of 0.5 pH unit, which took approximately 48 h. For solid medium 1 % (w/v) Ionagar no. 2 (Oxoid) was added.

* Present address: Salmonella Reference Laboratory, Central Public Health Laboratory, Colindale Avenue, London, NW9 5HT.

Table 1. *Sources of Mycoplasma strains used*

Species	Strain	Site of recovery	Source
<i>Mycoplasma pulmonis</i>			
(1) Established strains	Negroni	Tissue culture	R. J. Fallon
	Ash	Rat lung	D. G. ff. Edward
	Kon	Rat lung	R. M. Lemcke
	880	Tissue culture	W. H. Murphy
	M2	Mouse lung	R. M. Lemcke
	M50	Mouse lung	R. M. Lemcke
	M72L	Mouse	R. M. Lemcke
	63	Rabbit	G. E. Kenny
	47	Rabbit	G. E. Kenny
	Sabin type C	Mouse lung	J. Tully
	Cheng	Rat lung	R. M. Lemcke
(2) Freshly isolated strains	Strain R	Rat lung	A. Hill
	M61E	Mouse ear	A. Hill
	RBU	Rat nose	A. Hill
	BWIL	Rat nose	A. Hill
	1014NP	Mouse nasopharynx	A. Hill
	G-3	Rat lung	A. Hill
	MacIndoe	Rat lung	A. Hill
	Strain G	Rat	A. Hill
<i>M. neurolyticum</i>	KSA	—	NCTC*
<i>M. arthritidis</i>	Campo	—	D. G. ff. Edward
<i>M. hominis</i>	PG21	—	Burroughs Wellcome
<i>M. fermentans</i>	PG18	—	Burroughs Wellcome

* National Collection of Type Cultures.

The organisms were harvested at 33400g for 30 min and then washed three times in phosphate buffered saline, each time being centrifuged at 46500g for 20 min. The protein content of the suspension was estimated by the method of Lowry, Rosebrough, Farr & Randall (1951).

Extraction. The organisms were sedimented at 46500g for 20 min and 0.2 ml phenol-acetic acid-water (2:1:0.5, w/v/v) mixture was added for each mg of protein in the pellet, mixed by pipette at intervals over a period of 30 min at room temperature and held overnight at 4 °C. After centrifugation at 46500g for 15 min, the extract, which contained 2 to 4 mg protein/ml, was stored in screw-capped vials at 4 °C.

Polyacrylamide-gel electrophoresis. The disc electrophoresis apparatus used as P.A.G.E. model 8-PE34 (35/S) (Quickfit and Quartz Ltd, Stone, Staffordshire). The polyacrylamide gel contained 35 % (v/v) acetic acid, 5 M-urea and 7.5 % (w/v) acrylamide (Takayama, MacLennan, Tzagoloff & Stoner, 1966). The unpolymerized mixture was degassed under vacuum, and 1 ml placed in each 5 × 65 mm glass tube and overlaid with 75 % (v/v) acetic acid. Polymerization of the acrylamide was carried out at 37 °C for 45 min.

When the gels were firm the overlay and unpolymerized material were removed and the surface was washed with 75 % acetic acid. The tubes were fitted to the holder, each gel was overlaid with 0.08 ml of 75 % acetic acid, and the tubes were filled carefully with 10 % (v/v) acetic acid, which was also used in the upper and lower reservoirs of the apparatus. The clear extract (100 µl) was mixed with 50 µl of a 40 % (w/v) sucrose solution in 35 % (v/v) acetic acid and was applied to the gel from a micropipette. The sample was allowed to sink slowly through the buffer layers on to the gel surface, thus minimizing mixing with the buffer. The lower electrode served as the cathode. Electrophoresis was started with a total

Fig. 1. Electrophoretic patterns of cell proteins of (A) *Mycoplasma hominis* strain PT21, (B) *M. fermentans* strain PG18, (C) *M. neurolyticum* strain KSA, (D) *M. arthritidis* strain Campo, (E) *M. pulmonis* strain Negroni.

current of 2 mA for 5 min to settle the proteins at the gel surface and then the current was increased to 4 mA/tube and run for 3 h at room temperature.

The gels were removed from the tubes under water and were stained with 1 % (w/v) Amido Black 10B in 7 % (v/v) acetic acid for 1 h. The stain was removed; the gels were rinsed in tap water and held overnight in 7 % acetic acid which helped to fix the proteins in the gel. The background stain was removed electrolytically in 7 % acetic acid using a direct current of 10 mA/tube for 45 min. The destained gels could be kept for an indefinite period if sealed in 7 % acetic acid and stored away from daylight.

RESULTS

Factors which might cause variations in the electrophoretic patterns of different extracts of one strain were examined by testing the Negroni strains of *Mycoplasma pulmonis* under the following conditions.

(1) Several batches, using stock cultures frozen on different dates as seed, were processed and the extracts were compared in one experiment.

Fig. 2. Electrophoretic patterns of cell proteins of *Mycoplasma pulmonis* strains representing (A) Group 1 - strain Ash, (B) Group 2 - strain 1014NP, (C) Group 3 - strain Sabin type C, and of individual strains, (D) strain M2, (E) strain 88o, (F) strain RBU, (G) strain Kon.

(2) Several extracts were made at one time from one batch of organisms and extracts were also made at intervals from another batch; the extracts were then compared.

(3) Extracts were compared from organisms which had been grown in media containing different batches of horse serum as the only variable, or in media with and without glucose.

(4) The patterns of extracts of organisms of different ages were compared; the patterns of extracts stored at 4 °C for different periods of time were also compared.

(5) Different clones (obtained by single colony subculture) originating from one frozen stock culture were compared.

(6) The electrophoretic patterns of one extract run on different days were compared.

There was no substantial difference in the patterns of the different extracts which were run on the same day. Conditions of growth and the age of the cells or extracts did not affect the electrophoretic patterns, thus confirming the findings of Razin & Rottem (1967) and Razin (1968).

Minor variations in the starting-point of the electrophoretic pattern and in the quantity of

protein applied to the gel occurred. Medium components contributed no lines to the electrophoretic pattern: uninoculated medium was incubated for several days, centrifuged, and the small pellet was extracted and subjected to electrophoresis but gave no lines when the gel was stained.

Electrophoretic patterns of different species. Several human species of mycoplasmas were extracted and compared with rodent strains (Fig. 1). Species differentiation was possible, each pattern being distinct, thus confirming the findings of Razin & Rottem (1967) and Haas, Sacks & Razin (1968).

Comparison of strains of Mycoplasma pulmonis. The electrophoretic patterns of 19 strains of *M. pulmonis* were compared after the organisms had been grown and extracted under standard conditions. The patterns were not identical. There was, in most cases, a 'basic *M. pulmonis* pattern', seen particularly in the first four to six lines near the top of the gel. Differences were more distinct in the patterns of some strains than of others; e.g. strain 880 and strain Kon had distinct patterns, but in the case of Kon the pattern of the top group of lines was not very close to the 'basic *M. pulmonis* pattern'.

Three groups of strains were observed (Fig. 2), with some individual strains. Most of the strains possessed electrophoretic patterns which resembled the 'basic *Mycoplasma pulmonis* pattern'. The patterns of several recent isolates differed from those of long adapted strains of *M. pulmonis* in being more densely stained and more clear-cut. Some had more bands in the pattern particularly in the lower region of the gel. Some recent isolates resembled the adapted strains closely while others were more distinct.

The groups of strains having similar patterns were: Group 1 - Negroni, M72L, Ash and 63; Group 2 - strain G, Cheng 47 clone T, 1014NP, BWIL, strain G-3; Group 3 - M61E, Sabin type C, Macindoe. The individual strains included Kon, 880, RBU, strain R, M2 and M50.

DISCUSSION

Only a few mycoplasmal species among those examined to date show significant or distinct differences between strains at the subspecies level. Intraspecies differences between strains of *Mycoplasma hominis* have been demonstrated by several serological techniques (Nicol & Edward, 1953; Taylor-Robinson *et al.* 1965; Purcell *et al.* 1967; Hollingdale & Lemcke, 1970). Diversity within the species has also been demonstrated by Somerson *et al.* (1966), who used a nucleic acid homology technique, and by Razin (1968) and Hollingdale & Lemcke (1970), who showed that the proteins of strains of *M. hominis* possessed differing patterns in polyacrylamide-gel electrophoresis. Serological evidence of heterogeneity in the species *M. pulmonis* was produced by Leach & Butler (1966), Fallon & Jackson (1967) and Deeb & Keeny (1967). The electrophoretic patterns of proteins of strains of *M. pulmonis* (Razin, 1968) indicated different patterns which might permit division of the species into subtypes. However, nucleic acid homology studies failed to distinguish between strains of *M. pulmonis* (Somerson, Reich, Chanock & Weissman, 1967). In the present investigation distinct differences were observed between some strains although all strains were identifiable as members of the one species with the exception of Kon, the pattern of which did not closely resemble the 'basic *M. pulmonis* pattern'. Three groups of strains could be distinguished and there were a number of separate strains which possessed the '*M. pulmonis* pattern' but whose overall pattern differed from those produced by other strains examined.

The groups of strains with similar electrophoretic patterns were not the same as those formed from the results of serological studies (Forshaw & Fallon, 1972). This is not unexpected since quite different systems were being analysed and accords with the findings of

Taylor-Robinson, Martin-Bourgon, Watanabe & Addey (1971) with T-strain mycoplasmas. More detailed consideration of these findings and their relationship to the classification of *Mycoplasma pulmonis* is made in the discussion of the accompanying paper (Forshaw & Fallon, 1972) in which the results of serological examination of *M. pulmonis* strains are presented.

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