Identification of *Mycoplasma* and Other Microorganisms by Polyacrylamide-Gel Electrophoresis of Cell Proteins

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The proteins of *Mycoplasma* cells of various species produce highly reproducible and species-specific electrophoretic patterns in polyacrylamide gels containing 5 M urea and 35% acetic acid. These electrophoretic patterns can be used for the rapid identification and classification of *Mycoplasma*. Preliminary results indicate that this method may also be used for the identification and classification of other microorganisms.

The present classification of bacteria (*Bergey's Manual of Determinative Bacteriology*) is based primarily on morphological, biochemical, and serological characteristics. Since usually only a few such characteristics, representing a minute fraction of the bacterial genome, are tested, they frequently fail to express true genetic relatedness of microorganisms (2). The classification of *Mycoplasma* is particularly difficult, because, in the absence of a cell wall, their morphology is liable to great variations and their biochemical activity is sluggish. The classification is therefore based largely on their serological behavior (6). Recent deoxyribonucleic acid (DNA) base composition and nucleic acid hybridization techniques are the most direct means of ascertaining genetic relatedness of microorganisms, and have been successfully applied to the taxonomy of mycoplasmas (6). However, the routine laboratory is not equipped to carry out these techniques which are still too complex for large-scale comparative studies even by the specialized laboratory. Thus there is an obvious need for a simple method to indicate the genetic relatedness of microorganisms.

In studying the electrophoretic behavior of *Mycoplasma* membrane proteins in polyacrylamide gels containing acetic acid and urea, we noticed that the electrophoretic patterns obtained were highly reproducible and specific for the different *Mycoplasma* strains examined. Since there is little doubt that the synthesis of membrane proteins is genetically directed (9), the electrophoretic pattern of membrane proteins may well reflect genetic identity or nonidentity of the microorganisms. We proposed, therefore, that the electrophoretic patterns of membrane proteins

be used as "finger prints" for the identification of *Mycoplasma* (15). A great disadvantage of the proposed method is the use of cell membranes, calling for a relatively large quantity of cells and involving the tedious procedure of cell fractionation. The use of whole cells was therefore investigated. The results show that the electrophoretic patterns of whole cell proteins are adequate for strain identification, thus eliminating the need for isolated cell membranes.

MATERIALS AND METHODS

Organisms and growth conditions. M. arthritidis (PG6), M. hominis Type 2 (Campo, PG27), M. pulmonis (PG34), M. pulmonis (PG53, the Negroni agent), M. gallisepticum (PG31), M. maculosum (PG 15), M. canis (PG14), M. spumans (PG13), and *Mycoplasma* sp. strain C 21 were obtained from D. G. ff. Edward (The Wellcome Research Laboratories, Beckenham, Kent, England). M. hominis Type 2 strains O7 and H39 were obtained from P. F. Smith (The University of South Dakota, Vermillion). The M. hominis Type 2 strains have been recently reclassified as M. arthritidis (5). The organisms were grown in 100 to 200 ml of broth (1) for 24 to 48 hr at 37 C. The cells were harvested by centrifugation at 13,000 × g for 10 min, washed twice in 0.25 M NaCl, and suspended in 1 ml of the NaCl solution. The amount of cell protein in the suspension was determined according to Lowry et al. (13).

Polyacrylamide gel electrophoresis. The gels contained 7.5% (w/v) acrylamide, 35% (v/v) acetic acid, and 5 M urea in 6 mm × 100 mm glass tubes (17). For the 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 N,N'-methylenbisacrylamide in 47% (v/v) acetic acid were 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 overlayered with 75% (v/v) acetic acid. Polymerization of the acrylamide was carried out at 37 C for 45 min. The cells were dissolved by adding two volumes of phenol-acetic acid-water (2:1:0.5, w/v/v) to one volume of cell suspension. The insoluble material was removed by centrifugation at 30,000 × g for 15 min. The clear supernatant fluid (100 μliters containing 200 to 400 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. The 75% (v/v) acetic acid (0.5 ml) was then 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. Destaining was done electrolytically in 7% (v/v) acetic acid by using a direct current of 200 ma for 2 hr.

RESULTS AND DISCUSSION

The phenol-acetic acid-water mixture was found to be very effective in solubilizing the hydrophobic or structural membrane proteins (15, 17) and probably also the ribosomal proteins. Thus most of the structural cell proteins are dissolved. The acetic acid and urea in the gels keep the proteins in their monomeric form and prevent reaggregation, thus assuring reproducible electrophoretic patterns (17).

Some of the electrophoretic patterns of the many Mycoplasma strains examined were selected to illustrate the merits of the new method. The first group consists of the electrophoretic patterns of the rat pathogen M. arthritidis strain PG6 and the strains Campo (PG27), H39, and O7 isolated from the human genital tract and known as M. hominis Type 2. Recent serological and nucleic acid homology studies have indicated that M. hominis Type 2 strains are closely related or identical with M. arthritidis, and it has been suggested that the classification of Mycoplasma strains as M. hominis Type 2 should be withdrawn (5, 11, 12, 16). The electrophoretic patterns in Fig. 1 show the close resemblance of M. hominis Type 2 strains to M. arthritidis, and justify their inclusion in one species.

Another example involves the Mycoplasma strain which was isolated from tissue cultures inoculated with bone marrow from leukemia patients, and is known as the Negroni agent (8, 14). By serological, biochemical, and nucleic acid homology techniques (7, 10, 16) this Mycoplasma was shown to be closely related to the rat pathogen M. pulmonis. Again, the close similarity of the electrophoretic patterns of these strains corro-

borates the identification of the Negroni agent as a M. pulmonis strain (Fig. 2).

The electrophoretic method also proved to be very useful in identifying fresh isolates from clinical material. Nasal and throat smears from a dog suffering from pneumonia gave rise to numerous Mycoplasma colonies on agar medium. On the basis of colony morphology, three different types could be cloned. By comparison of the electrophoretic patterns of the unknown clones with those of the three established canine Mycoplasma species (3, 4), one of our isolates could be identified as M. spumans (Fig. 3) and the other closely resembled M. maculosum. The third strain did not correspond with any of the established canine strains and was the only Mycoplasma to be isolated from the dog's lung after its death. The electrophoretic pattern of this strain was identical to that of Mycoplasma sp. strain C21 isolated from the throat of a dog by Edward and Fitzgerald about 17 years ago (3).

So far, the experience with the new method indicates that different growth conditions do not significantly affect the electrophoretic patterns. Thus, a change in the basal medium, or in the concentration or type of serum included in the growth medium, did not change the characteristic electrophoretic pattern of M. gallisepticum proteins. Furthermore, the age of the cells did not affect the pattern (Fig. 4). However, we recommend that the strains to be compared should be grown under identical conditions.

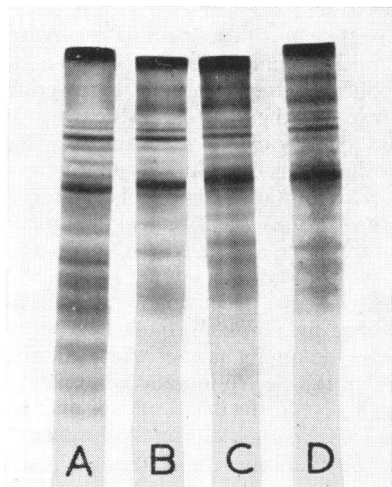The electrophoretic method was also successfully applied to the identification of the stable L

FIG. 1. Electrophoretic patterns of cell proteins of: (A) M. arthritidis (PG6); (B) M. hominis Type 2 (Campo, PG27); (C) M. hominis Type 2 (07); and (D) M. hominis Type 2 (H39).
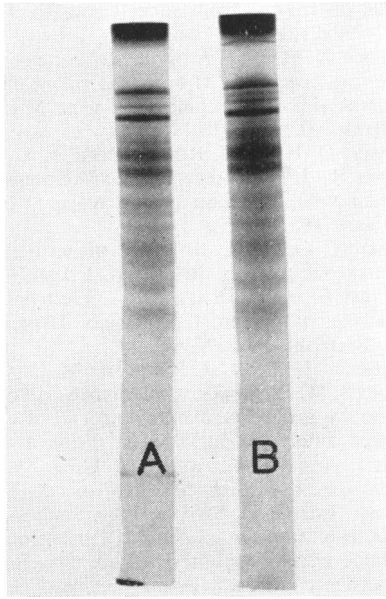
FIG. 2. *Electrophoretic patterns of cell proteins of: (A) M. pulmonis (PG34) and (B) the Negroni Mycoplasma (PG53).*
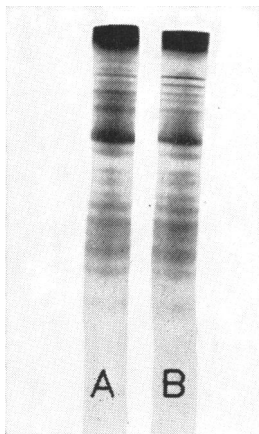

FIG. 3. *Electrophoretic patterns of cell proteins of: (A) M. spumans and (B) a Mycoplasma isolated from the nose of a dog suffering from pneumonia.*

FIG. 4. *Electrophoretic patterns of cell proteins of Mycoplasma gallisepticum grown in: (A) broth containing 20% (v/v) horse serum; (B) broth containing 2% (v/v) PPLO Serum Fraction (Difco), cells harvested after 24 hr of incubation; and (C) broth containing 2% (v/v) PPLO Serum Fraction, cells harvested after 48 hr of incubation.*

forms of *Streptobacillus moniliformis* and *Proteus vulgaris*. Each of the bacterial L forms yielded a reproducible and characteristic electrophoretic pattern. Preliminary experiments have also shown characteristic electrophoretic patterns of enteric bacteria cell proteins.

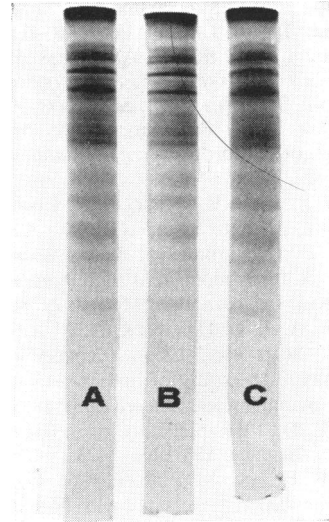The small quantity of cells required for the test (less than 1 mg of cell protein), the wide availability of electrophoresis equipment, elimination of the need for expensive antisera, and the rapidity of the test should favor the wide application of the proposed method.

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