Electrophoretic Analysis of Proteins from *Mycoplasma hominis* Strains Detected by SDS-PAGE, Two-dimensional Gel Electrophoresis and Immunoblotting

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The proteins of 14 strains of *Mycoplasma hominis* were compared by SDS-PAGE in gradient gels, by two-dimensional (2D) gel electrophoresis of extracts of ^{35}S -labelled cells and by immunoblot analysis of cell proteins. The strains examined included the *M. hominis* type strain PG21 and 13 others isolated variously from genital tract, mouth, blood, upper urinary tract and a wound. These 14 strains shared 76–99% of proteins in SDS-gradient gel analysis and 41–72% in the 2D gels. As expected, the immunoblot analysis likewise revealed the existence of an extensive common protein pattern in *M. hominis*, in addition to a number of antigens shared only by some strains.

INTRODUCTION

There are 12 species of the class *Mollicutes* currently recognized as occurring in man, four of which are associated with the genital tract – *Mycoplasma hominis*, *Mycoplasma fermentans*, *Mycoplasma genitalium* and *Ureaplasma urealyticum*. *M. hominis* is commonly found in the female genital tract and several epidemiological, microbiological, serological and animal model studies have shown that it is potentially pathogenic and may be involved in, for example, acute pelvic inflammatory disease (Taylor-Robinson & McCormack, 1980), acute pyelonephritis (Thomsen, 1978) and postpartum fever (Platt *et al.*, 1980). However, assessment of the pathogenic potential of *M. hominis* is complicated by the fact that this species constitutes a relatively heterogeneous group of organisms. Serologically, significant antigenic differences have been demonstrated by tube agglutination (Nicol & Edward, 1953), indirect haemagglutination (IHA) (Taylor-Robinson *et al.*, 1965; Hollingdale & Lemcke, 1970; Thomsen, 1978), metabolism inhibition (MI) (Purcell *et al.*, 1967; Hollingdale & Lemcke, 1970; Lin & Kass, 1974; Thomsen, 1978) and a complement-dependent mycoplasmacidal test (MC) (Lin & Kass, 1974). By growth inhibition (GI) intraspecific differences were found by Razin (1968) and Lin *et al.* (1975), but not by Hollingdale & Lemcke (1970). Unpublished studies in our laboratory did not reveal any intraspecific heterogeneity by GI, whereas various degrees of cross-reactivity between a total of 14 *M. hominis* strains (those examined in the present study) were obtained by IHA, MI and MC tests. In complement fixation (CF) tests, little difference between strains was found by Hollingdale & Lemcke (1970). Razin (1968) and Hollingdale & Lemcke (1970) found dissimilarities in the protein patterns of different *M. hominis* strains by PAGE. Genetic heterogeneity within the species has been demonstrated by nucleic acid hybridization studies (e.g. Somerson *et al.*, 1966; Barile *et al.*, 1983).

Abbreviations: 2D, two-dimensional; CF, complement fixation; GI, growth inhibition; IHA, indirect haemagglutination; MC, mycoplasmacidal; MI, metabolism inhibition.

The objective of the present investigation was to determine whether this reported intraspecific heterogeneity of *M. hominis* could be confirmed and analysed by examining the protein patterns of 14 strains, using SDS-PAGE, two-dimensional (2D) gel electrophoresis and immunoblotting. The 2D gel electrophoresis technique was included because of its known usefulness in detecting differences in protein patterns between relatively closely related mycoplasmas (Rodwell & Rodwell, 1978; Mouches *et al.*, 1979; Rodwell, 1982; Andersen *et al.*, 1984; Nascimento *et al.*, 1986).

METHODS

Strains and cultivation. The source and origin of the 14 strains of *M. hominis* investigated are shown in Table 1. The strains include several extensively analysed by others (for references see Table 1). All strains were subcloned by filter (450 nm) cloning in the approved manner (International Committee on Systematic Bacteriology Subcommittee on the Taxonomy of Mollicutes, 1979). Their identity as *M. hominis* was confirmed serologically by growth inhibition, metabolism inhibition, indirect immunofluorescence and indirect haemagglutination tests.

Media. The mycoplasma strains, which are arginine-metabolizing and glucose-nonfermenting, were cultivated in a medium (BEA) supplemented with arginine and composed of heart infusion broth (Difco), 2.2% (w/v); horse serum, 15.5% (v/v); fresh yeast extract (Hayflick, 1965), 1.9% (w/v); thallium acetate, 0.008%; benzylpenicillin, 40 i.u. ml⁻¹; L-arginine, 0.23% (w/v) and phenol red, 0.0023% (w/v). The pH was adjusted to 7.2 and the medium sterilized by filtration.

SDS-PAGE of cell proteins. The mycoplasmas, unlabelled or labelled with [³⁵S]methionine, were freeze-thawed four times, resuspended in SDS sample buffer (0.0625 M-Tris/HCl pH 6.8, 2.3% (w/v) SDS, 5% (v/v) 2-mercaptoethanol, 10% (v/v) glycerol, 0.05% bromophenol blue), and heated to 100 °C for 2 min. Insoluble material was removed by centrifugation (2 min at 10000 g) in a microcentrifuge before electrophoresis was carried out in a 12–20% (w/v) polyacrylamide gradient gel. Samples (5–30 µl) containing about 30000 c.p.m. were applied to the gels. For unlabelled samples, the protein bands were visualized by Coomassie blue staining.

2D gel electrophoresis of cell proteins. The [³⁵S]methionine-labelled mycoplasmas were freeze-thawed four times and resuspended in lysis buffer as previously described (Andersen *et al.*, 1984). Samples containing 50000 c.p.m. were applied to a cylindrical pH 5–7.5 isoelectric focusing gel (165 × 2 mm) for separating acidic proteins (O'Farrell, 1975). The gels were run for 18 h at 400 V followed by electrophoresis in the second dimension (Laemmli, 1970). *M.* standards used in the second-dimension SDS-slab gel electrophoresis were [¹⁴C]methylated proteins (Amersham).

Measurement of the pH gradient in isoelectric focusing gels. One of the isoelectric focusing gels was cut into 5 mm slices and placed in 1 ml 0.1 M-KCl, and the pH was measured after the gel slices had been eluted (Archer *et al.*, 1978).

Preparation of rabbit hyperimmune serum. The antigens used for immunization of rabbits were prepared from organisms grown in rabbit-meat infusion broth and rabbit serum. (Taylor-Robinson *et al.*, 1963). Mycoplasma cultures grown in 600 ml RE-medium (Ernø *et al.*, 1973), supplemented with 1% (v/v) PPLO serum fraction (Difco) were harvested in the exponential phase, by centrifugation at 38000 g for 1 h, washed twice in PBS buffer (12 mM-sodium phosphate, 0.145 M-sodium chloride, pH 7.0), and resuspended in 24 ml PBS. After sonication (Branson sonifier B-12; 1 min at 40 W) the antigen was stored in 1 ml volumes at –20 °C. Two rabbits were inoculated intramuscularly twice a week, for three weeks, with a mixture of equal volumes (0.75 ml) of antigen and Freund's complete adjuvant. Three weeks later they were given three injections (every second day) of antigen (1.5 ml) in the marginal ear vein. Two to four weeks after the last injection, blood was obtained and tested for reactivity by immunofluorescence. The rabbits were killed by exsanguination and the sera stored in 5 ml volumes at –20 °C. Preimmunization sera were obtained from all the rabbits and included as controls in every test.

Immunoblotting. SDS-PAGE for immunoblotting was done using the discontinuous buffer system (Laemmli, 1970) in a Bio-Rad protein dual-slab cell. Mycoplasma proteins, prepared as described for SDS-PAGE, were applied (approx. 5 µg protein per track) to a 10% (w/v) polyacrylamide gel (final dimensions 160 × 130 × 0.8 mm). Proteins were transferred from the SDS-PAGE slab gel to nitrocellulose membranes (Schleicher & Schuell BA-85, pore size 0.45 µm) by means of a Bio-Rad trans-blot cell. The electrode buffer (transfer buffer) was 0.025 M-Tris, 0.192 M-glycine (pH 8.3). A voltage of 15 V cm⁻¹ was applied for 4 h (current 0.34–0.75 A). The temperature was kept between 4 °C and 8 °C. Two different methods were used to detect antigens transferred to nitrocellulose.

Method (1) (Bjerrum *et al.*, 1983). After transfer, the remaining sites for protein binding were blocked by incubating the nitrocellulose blots for 2 min at room temperature in TBS buffer (50 mM-Tris, 150 mM-NaCl, 2%, w/v, Tween 20, pH 10.2). The blots were then washed three times in TTBS buffer (50 mM-Tris, 150 mM-NaCl, 0.05%, w/v, Tween 20, pH 10.2), in order to remove excess blocking agent, before incubation with gentle rocking for 30 min at 37 °C with primary antibody (rabbit antiserum) diluted 1 in 100 in TTBS buffer. Unbound antibody was removed by washing the blots three times for 10 min in TTBS. Incubation with secondary antibody (goat anti-

Table 1. *Mycoplasma hominis* strains and their origin

Strain	Source of isolation	Reference
PG21 (H50)*	Lower genital tract	Nicol & Edward (1953)
M1449	Blood	Friis <i>et al.</i> (1983)
93	Vagina	Lin & Kass (1974)
10		
4195		
183		
132		
W2	Wound	Lee <i>et al.</i> (1971)
SC4	Male urethra	Hollingdale & Lemcke (1969)
DC63	Oral cavity	Taylor-Robinson <i>et al.</i> (1963)
V2785		
P71	Upper urinary tract	Thomsen (1978)
P7		
P2		

* Type strain.

rabbit immunoglobulin-peroxidase conjugated, Bio-Rad) was performed as described above, but with a working dilution of 1 in 3000. The blots were washed again and then immersed in a freshly prepared staining solution containing 50 ml 50 mM-sodium acetate, with 0.175% (v/v) acetic acid (pH 5.5); 20 mg 3,3'-diaminobenzidene-tetrahydrochloride (peroxidase substrate) and 25 μ l H₂O₂ (30%, w/v). The staining was complete within 1–5 min. The developed blots were washed in distilled water for 10 min and incubated in 50 mM-sodium disulphite for another 5 min. The blots were then dried.

Method (2) (Bio-Rad Instruction Manual). Blocking was effected by treatment for 10 min at 37 °C with 3% (w/v) gelatin, 20 mM-Tris, 500 mM-NaCl, pH 7.5. The blots were washed three times in 20 mM-Tris, 500 mM-NaCl, 0.05%, w/v, Tween 20, pH 7.5. Primary antibody, diluted 1 in 50 in 1% (w/v) gelatin in 20 mM-Tris, 500 mM-NaCl, pH 7.5, was applied and the test incubated at 37 °C for 1 h. Washing was done as previously indicated. The secondary antibody was applied at a dilution of 1 in 3000 in the same buffer as for primary antibody, and incubated at 37 °C for 1 h. The blots were washed as before but with one additional washing in 20 mM-Tris, 500 mM-NaCl, pH 7.5, because Tween 20 interferes with the staining reaction using 4-chloro-1-naphthol. The staining solution was 20 mM-Tris, 500 mM-NaCl, 17% (v/v) methanol, 0.4 mg 4-chloro-1-naphthol ml⁻¹; staining was complete within 15–30 min. The blots were washed in distilled water and dried.

RESULTS

The proteins of the 14 *M. hominis* strains proved very similar when analysed in SDS-gradient gels. At least 70 distinct protein bands were seen in autoradiograms as well as by staining of unlabelled proteins with Coomassie blue. A comparative analysis of the protein bands obtained with the 14 strains revealed very similar electrophoretic patterns, with similarities between 76% and 99% congruence. The results obtained for some of the strains are shown in Fig. 1(a), including the two most similar strains (DC63 and 132, with 99% congruence) and the two most different strains (P7 and V2785, with 76% congruence). In spite of the high similarity values, it was possible to distinguish each of the 14 strains on the basis of small differences in the protein patterns, as demonstrated in Fig. 1(b), showing, for four strains, an enlarged segment of the autoradiogram in Fig. 1(a). Dissimilar bands in the 14 strains were most evident in the M_r 35000, 44000, 78000 and 106000 regions. Comparative gradient gel analysis between the type strain PG21 and the other 13 *M. hominis* strains showed 78–87% congruence.

Autoradiograms of the 2D gels from the 14 *M. hominis* strains were exposed for about four weeks. Between 54 and 94 acidic proteins were detected in each autoradiogram and further exposure revealed no more. The basis for comparisons of the autoradiograms was M_r standards in the second dimension and determination of the pH gradient in the focusing dimension. Each autoradiogram was divided into squares according to specific pH and M_r values as previously described (Andersen *et al.*, 1984). The electrophoretic 2D protein patterns of the 14 *M. hominis* strains had similarities between individuals ranging from 41% to 72% (Table 2) as derived from the number of common protein spots and calculated from the formula previously described

Fig. 1. (a) SDS-polyacrylamide gradient gel electrophoretic patterns of ^{35}S -labelled cell proteins from seven of the 14 *M. hominis* strains. The electrophoresis was done for 16 h at 13 mA on a 12–20% SDS-polyacrylamide gradient gel. The fluorogram was exposed for 14 d. (b) An enlargement of an area of the gel in (a). Strains DC63 and 132 are almost identical, only one difference being demonstrable (arrows). In strain V2785 one band is missing, whereas two deviating bands are seen in M1449. M_r standards, indicated on the right, were [^{14}C]methylated proteins: myosin, M_r 200000; phosphorylase *b*, M_r 92000; bovine serum albumin, M_r 69000; ovalbumin, M_r 46000; carbonic anhydrase, M_r 30000 and lysozyme, M_r 14300. ([^{14}C]Methylated phosphorylase *b* may show two bands of M_r 92000 and 100000.)

(Rodwell & Rodwell, 1978; Rodwell, 1982; Andersen *et al.*, 1984). Most proteins were localized around pH 5–7 with M_r values between 30000 and 100000. When the 2D gels were compared, common proteins among all 14 *M. hominis* strains were found mainly in the M_r range 25000–60000. Thirty-two of the quantitatively most prominent protein spots, all present in strain PG21 (Fig. 2a), were selected for a gel spot comparison. Sixteen of these selected proteins were shared by all the strains. The results of the comparisons are shown in Table 3. An example of the gel spot comparison is given in Fig. 2(b), showing proteins common to strains P71 and PG21. Thirty-eight of the protein spots were shared by the two strains and the calculated percentage congruence was 58% (Table 2).

Heterogeneity of immunogens was studied by two different immunoblot analyses. Coomassie blue stained SDS-PAGE patterns of cell proteins from the 14 *M. hominis* strains showed between 45 and 55 separate bands (Fig. 3), of M_r 19000–200000. By the first immunoblot analysis (Bjerrum *et al.*, 1983), proteins from all 14 strains were transferred to nitrocellulose and tested against each of the corresponding 14 rabbit hyperimmune sera. An example is shown in Fig. 4, which gives the results obtained when antiserum to strain M1449 was tested against all 14 strains. By the second immunoblot analysis (Bio-Rad), proteins from each of the 14 strains were transferred to nitrocellulose, sliced and tested against each of the 14 rabbit hyperimmune sera. The results obtained for the proteins prepared from strain DC63 are shown in Fig. 5. A complete cross-examination of the 14 strains was made twice by each method; it produced results very

Table 2. *Protein homologies of 14 M. hominis strains*

Protein homologies are given as the number of common protein spots found by comparing autoradiograms of 2D gels of the 14 strains, and as percentage congruence (in parentheses), calculated from the formula $[2N_{AB}/(N_A + N_B)] \times 100$, where N_A and N_B are the total number of spots on the fluorograms for strain A and B respectively, and N_{AB} is the number of common spots (Rodwell & Rodwell, 1978).

	DC63	V2785	M1449	SC4	183	132	W2	10	93	4195	P2	P7	P71	PG21
DC63	68 (100)													
V2785	35 (51)	69 (100)												
M1449	36 (54)	42 (62)	66 (100)											
SC4	45 (63)	40 (55)	41 (58)	76 (100)										
183	30 (46)	29 (44)	28 (44)	35 (51)	62 (100)									
132	40 (54)	36 (48)	40 (54)	42 (54)	34 (48)	81 (100)								
W2	34 (54)	36 (56)	40 (64)	39 (58)	34 (56)	37 (53)	59 (100)							
10	30 (46)	35 (53)	36 (56)	33 (47)	33 (53)	34 (47)	44 (72)	63 (100)						
93	42 (61)	45 (65)	40 (59)	44 (60)	35 (53)	49 (65)	38 (59)	33 (50)	70 (100)					
4195	50 (62)	36 (44)	36 (45)	42 (50)	34 (44)	49 (56)	43 (57)	32 (41)	42 (52)	93 (100)				
P2	40 (54)	43 (58)	42 (58)	45 (58)	33 (47)	51 (64)	41 (59)	32 (45)	46 (62)	50 (58)	79 (100)			
P7	35 (57)	35 (57)	39 (65)	35 (54)	34 (59)	37 (55)	30 (53)	30 (51)	37 (60)	33 (42)	43 (65)	54 (100)		
P71	39 (57)	40 (58)	34 (50)	41 (56)	29 (44)	40 (53)	42 (65)	36 (54)	41 (59)	44 (54)	45 (60)	42 (68)	70 (100)	
PG21	34 (53)	38 (59)	29 (46)	37 (54)	26 (43)	37 (52)	29 (49)	33 (54)	36 (55)	32 (42)	33 (47)	34 (60)	38 (58)	60 (100)

Fig. 2. (a) Diagrammatic representation of the positions of proteins as seen in 2D gel electrophoresis of cell proteins of *M. hominis* strain PG21 (type strain). Thirty-two of the quantitatively most prominent proteins are shown. (b) Positions of proteins common to *M. hominis* strains P71 and PG21, as seen when comparing the 2D electrophoretic protein patterns of cell proteins. Sixteen of the quantitatively most prominent proteins also shared by the other 12 *M. hominis* strains are indicated by arrows. M_r standards (see Fig. 1) are shown to the left, and the pH gradient is shown at the top of each diagram.

Table 3. Occurrence of proteins present in *M. hominis* strains as shown by 2D gel spot comparisons

Each column indicates the presence (+) or absence (0) of each of the 32 selected spots that were all present in the autoradiogram for the type strain, PG21, which was used as the standard for comparisons with that for each other strain.

PG21 spot no. compared*	DC63	V2785	M1449	SC4	183	132	W2	10	93	4195	P2	P7	P71
1	+	+	+	+	+	+	+	0	+	0	+	0	+
3	+	+	+	+	+	+	+	+	+	+	+	+	0
4	+	+	0	+	+	+	+	0	+	+	+	+	+
5	+	0	0	+	+	0	+	+	+	+	0	0	0
7	+	+	0	+	+	+	+	0	+	+	+	+	+
8	+	+	0	+	0	0	0	0	+	+	+	0	+
10	+	0	+	+	+	+	+	+	+	0	+	+	+
11	+	0	+	+	+	+	+	+	+	0	+	+	+
13	+	0	0	0	0	0	0	0	0	0	0	0	0
19	+	+	+	+	+	+	+	+	+	+	0	0	0
20	+	+	+	+	+	+	+	+	+	+	0	0	+
24	+	+	+	+	+	+	+	+	+	+	+	0	+
25	0	0	+	+	0	+	+	+	+	+	+	+	+
26	0	+	0	0	+	0	0	0	0	0	+	+	0
27	0	+	0	+	+	+	+	+	+	+	+	+	+
29	+	+	+	+	+	+	0	+	+	+	+	+	+

* Spot numbers not listed were present in all the strains.

similar to those shown in Figs 4 and 5. Using each of the 14 rabbit hyperimmune sera it was possible to identify 20–29 individual protein immunogens in the 14 strains, with M_r values ranging between 30000 and 100000. Nineteen prominent proteins, indicated by numbers in Figs 3, 4 and 5, were selected for a more detailed study (Table 4). Common bands were most evident in the M_r regions 30000–39000, 44000–57000 and 92000–96000. The comparisons showed that 12 of the 19 antigens were found in all strains (Table 4). Eight of these antigens (2, 3, 11, 12, 16, 17, 18 and 19) were recognized when testing all 14 hyperimmune sera against any of the 14 *M. hominis* strains with the method described by Bjerrum *et al.* (1983) (with the Bio-Rad method, bands 16, 17, 18 and 19 were sometimes faint or undetectable). Antigen 1 was found in all but two (V2785 and PG21) of the 14 strains. However, antiserum against each of these two strains

Fig. 3. SDS-PAGE patterns of cell proteins from 14 *M. hominis* strains. The electrophoresis was done for 16 h at 60 V on a 10% (w/v) polyacrylamide gel. The gel was stained with Coomassie blue. The positions of the M_r standards are shown on the left. The numbers to the right indicate some proteins detected by immunoblotting analysis (Figs 4 and 5); the M_r range is from 102 000 to 19 000 (bands 1 and 22). M_r standards: phosphorylase *b*, M_r 94 000; bovine serum albumin, M_r 67 000; ovalbumin, M_r 43 000; carbonic anhydrase, M_r 30 000 and soybean trypsin inhibitor, M_r 20 100.

did react with antigen 1 in all the other strains, except strain 10. Therefore, strains V2785 and PG21 apparently carried this antigenic determinant, but on a protein of different M_r . Antigens 11, 12 and 13/14 were quantitatively the most dominant bands seen in SDS-PAGE, as well as in the immunoblotting with both homologous and heterologous hyperimmune sera. Seven of the 19 antigens were not found in all 14 *M. hominis* strains (Table 4). Antigen 14 was found only in those strains that did not have antigen 13. Therefore, these two antigens seemed to be immunologically identical and could be recognized by all 14 *M. hominis* strain hyperimmune sera. More complex reaction patterns were seen in the region M_r 70 000–90 000, where the bands seemed to be very different for all 14 strains. Immunological detection of minor antigens in this region showed a greater variation than could be seen by SDS-PAGE. An example is the antigen band 6 found by SDS-PAGE in all strains. This protein was detected in immunoblotting tests by all homologous sera, but showed a marked variation with heterologous sera, which indicated that the protein was variable.

DISCUSSION

According to the definition proposed by the International Committee on Systematic Bacteriology Subcommittee on the Taxonomy of *Mollicutes* (1979), a mycoplasma species may, ideally, be regarded as a cluster of morphologically similar isolates that exhibit a high degree of

Fig. 4. Immunological identification of *M. hominis* antigens after blot transfer using a rabbit antiserum to *M. hominis* M1449. Some of the 22 protein bands selected from the SDS-gel (Fig. 3) and which were immunologically detected are shown to the right. The method used was according to Bjerrum *et al.* (1983). M_r standards as in Fig. 3.

genetic and phenotypic relatedness. However, the degree of intraspecific homogeneity actually found in individual species of the mollicutes varies widely. *M. hominis* is clearly among the species which exhibit particularly marked intraspecific heterogeneity (see Introduction). The complexity of the relationships based upon serological differences between strains of this species was emphasized by Hollingdale & Lemcke (1969), who showed that antigens stimulating the development of GI, MI and IHA antibodies resided almost exclusively in the cell membrane, whereas antigens involved in the CF test were mainly associated with the cytoplasmic cell fraction.

In the following discussion of our results, it should be kept in mind that the immunizing antigens used for production of antisera were prepared from sonically disrupted mycoplasma cells. Similarly, the protein preparations used in PAGE, 2D gel electrophoresis, and immunoblotting experiments were lysates prepared from whole-cell suspensions. Hence, the results obtained do not discriminate between membraneous and cytoplasmic proteins. The two immunoblotting methods we used were chosen because they allow a direct comparative analysis of those parts of the protein patterns that are responsible for antigenic similarities and dissimilarities between different strains, as revealed by conventional serology, and these results can also be directly compared with those obtained by SDS-PAGE. All these methods have the limitation, however, that only a very minor proportion of the cell proteins is detected. For example, the number of proteins recognized by 2D gel electrophoresis (between 54 and 93) in different strains represents only 8–15% of that expected from the coding capacity of the genome (Andersen *et al.*, 1984). However, it is noteworthy that the average number of 70 distinct bands demonstrated in this study by SDS-PAGE, whether in autoradiograms (Fig. 1) or by Coomassie

Fig. 5. Immunological identification of antigens of *M. hominis* strain DC63 after blot transfer using rabbit antisera against 14 *M. hominis* strains. Some of the 22 protein bands selected from the SDS-gel (Fig. 3) and which were immunologically detected are shown to the right. The method used was according to the Bio-Rad standard procedure. M_r standards as in Fig. 3.

Table 4. *Results of an immunoblotting analysis of 14 M. hominis strains*

Nineteen protein antigens found in SDS-PAGE (see Figs 3-5) were selected for this investigation.

Antigen	M_r	No. of strains in which antigen found in SDS-PAGE	Strains in which antigen not found	Antigens detected by all 14 antisera
1	102000	12	V2785, PG21	
2	96000	all		×
3	94000	all		×
4	92000	13	4195	×
5	88000	all		
6	83000	all		
7	78000	8	DC63, V2785, W2, 10, 93, 4195	
8	68000	all		
9	58000	11	DC63, SC4, 132	
10	57000	9	V2785, M1449, 183, W2, 10	
11	49000	all		×
12	47000	all		×
13	44000	10	DC63, V2785, SC4, 132	×
14	43000	4	All except DC63, V2785, SC4, 132	×
15	39000	all		
16	38000	all		×
17	33000	all		×
18	31000	all		×
19	30000	all		×

blue staining of unlabelled proteins, greatly exceeds the numbers obtained in earlier studies (Razin, 1968; Hollingdale & Lemcke, 1970); this increase in resolution reflects technical improvements in the PAGE technique. Furthermore, the bands detected in our immunoblot assay were much more numerous than those found by Brown *et al.* (1983) in their Western blot analysis of the seven strains of Lin & Kass (1974) with an *M. hominis* PG21 rabbit antiserum. As that serum, in contrast to our hyperimmune sera, was produced by immunization with (presumably relatively intact) whole cells, the antigens detected by Brown *et al.* (1983) were probably mainly confined to the cell surface. No other studies of *M. hominis* by 2D gel electrophoresis are available for comparison with ours.

In general, the results of our comparative assay of 14 selected strains of *M. hominis* confirms and extends previous observations by other workers of a relatively high degree of intraspecific heterogeneity. The finding of apparently more pronounced similarities between the strains when tested by SDS-PAGE (range of congruence 76–99%), as compared to the results of 2D gel electrophoresis (calculated homology 41–72%) is not surprising: since 2D gel electrophoresis separates individual bands according to both their isoelectric point and their M_r , this method is likely to provide a higher degree of resolution.

A study by Barile *et al.* (1983) indicated that strains of *M. hominis* isolated from the same source, such as post-partum blood culture, the urogenital tract, or cell cultures, have very high genomic homology, strains from each source falling into clusters. Although the 14 strains we examined were derived from varied sources (Table 1), the number of strains of identical origin was in most cases not sufficiently high to allow any valid conclusions. Only three strains (P2, P7 and P71) isolated from one site (the upper urinary tract of patients with acute pyelonephritis) appeared to be particularly similar, although not greatly different from other strains. It is worth mentioning that the seven strains isolated from the lower urogenital tract (Table 1) were not found to be more closely related to each other than to strains isolated from other locations. This apparent discrepancy between our findings and those reported by Barile *et al.* (1983) may indicate a lack of close correlation between nucleic acid homology and antigenic properties; it might also be explained, at least in part, by the antigenic drift that may occur during repeated passages of laboratory strains, a phenomenon that makes it difficult to compare data from different laboratories (M. F. Barile, personal communication).

The implications of the heterogeneity of *M. hominis* were discussed recently by Freundt (1983), with particular regard to the consequences it may have for identification of new isolates and for serodiagnosis of *M. hominis* infections, and to taxonomic considerations. As to the taxonomic status of *M. hominis*, the tentative proposal by Lin & Kass (1974) and Lin *et al.* (1975) to recognize at least seven serovars of the species has never received general acceptance. The observations presented in this paper do not, in our opinion, provide any additional evidence in support of that proposal.

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