Specific Protein Differences Among Strains of *Ureaplasma urealyticum* as Determined by Two-Dimensional Gel Electrophoresis and a Sensitive Silver Stain

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The proteins of nine strains of *Ureaplasma urealyticum*, representing serovars I through IX, were analyzed by using two-dimensional gel electrophoresis and a sensitive silver staining procedure. The electrophoretic patterns of the nine strains tested could be divided into two distinct major groups, one group consisting of serovars II, IV, V, VII, VIII, and IX (group A) and one group consisting of serovars I, III, and VI (group B).

Ureaplasma urealyticum strains are a serologically heterogeneous group of organisms (16) that are frequently found in human genital tracts. The pathogenic potential of these organisms has been widely disputed, and the significance of their isolation is often unclear (4, 17, 18). The possibility that some strains or biotypes of *U. urealyticum* are pathogenic and others are not has been suggested (15), but serological differences have not been clearly associated with pathogenicity (10, 15). Deoxyribonucleic acid hybridization (5) and one-dimensional (8, 13) and two-dimensional (9) polyacrylamide gel electrophoretic analyses of [³⁵S]methionine-labeled proteins have revealed differences among strains of *U. urealyticum*. In two of these studies (5, 9), the nine recognized serovars could be separated into two major groups. In this report we describe an additional method of analyzing the proteins of *U. urealyticum* strains by using two-dimensional electrophoresis and a sensitive silver-based stain which combines with proteins in a fashion that produces reproducible and characteristic colors (12). Our findings confirm previous reports of two groups within the species *U. urealyticum*.

Eight strains of *U. urealyticum* originally obtained from F. T. Black, Aarhus, Denmark, and designated serovars I through VIII (3) and one strain obtained from J. A. Robertson, Alberta, Canada, and designated serovar IX (11) were used. These organisms were grown in U-9 broth (14) supplemented with 2% PPLO serum fraction (Difco Laboratories, Detroit, Mich.) at 37°C in an atmosphere containing 5% CO₂. Cultures in the log phase of growth were harvested by

centrifugation at 12,000 × *g* for 1 h and washed three times with phosphate-buffered saline. The cell pellet obtained from 1 liter of a 20-h culture was sufficient for more than six analyses. Uninoculated medium was incubated and alkalized to the same degree as growing cultures and was then centrifuged and processed in the same manner. Each strain was grown and tested at least three times.

Each washed cell pellet was suspended in six times its volume of sample buffer containing 2% sodium dodecyl sulfate, 2% β-mercaptoethanol, and 1% cyclohexylaminoethanesulfonic acid (Calbiochem-Behring Corp., La Jolla, Calif.) (pH 9.5) and heated at 98°C for 10 min. Electrophoresis in the first dimension (isoelectric focusing) and the second dimension (sodium dodecyl sulfate slab gel electrophoresis) was performed by using the method of Anderson et al. (2), as described by Dunbar et al. (6). Cylindrical isoelectric focusing gels containing ampholines with a wide pH range (pH 3.5 to 10; LKB, Hicksville, N.Y.) were used. A 20- to 40-μl portion of the solubilized sample was applied to the gels and focussed for 16 h at 400 V and 25°C. After equilibration, the isoelectric focusing gels were applied to 10 to 20% gradient polyacrylamide gels prepared by using the DALT system (Electronucleonics, Inc., Oak Ridge, Tenn.) described by Anderson et al. (2). This system had the advantage that multiple slab gels could be cast and run simultaneously so that identical gel patterns were easily obtained. In addition, the gradient was easily altered to optimize the resolution of high- or low-molecular-weight proteins. Rat heart homogenate (7) was used for molecular weight standards. After electrophoresis, the gels were fixed and stained by the procedures of Sammons et al. (12). In some cases, a GEL-CODE silver staining kit (UpJohn Diagnostics, Kalamazoo, Mich.) was used.

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FIG. 1. Two-dimensional gel electrophoresis of uninoculated medium containing PPLO serum fraction: isoelectric focussing in the first dimension followed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis in 10 to 20% gradient gels in the second dimension. The major medium components are indicated (spots A through D). The arrow indicates the bands produced by β -mercaptoethanol.

Figure 1 shows the gel pattern obtained when the solubilized proteins of uninoculated medium were applied to the gel system used. The band running across the upper half of the gel (Fig. 1, arrow) is an artifact due to the presence of mercaptoethanol in the equilibration buffer and occurs at a position corresponding to a molecular weight of 69,000. The vertical streak on the left (acidic) side of the gel was due to the presence of sodium dodecyl sulfate in the sample. Spot A may represent serum albumin (1, 6, 12). The chain of spots labeled B stained red, whereas the cluster of spots labeled C stained yellow. The large, diffuse spot at an apparent molecular weight of 22,000 to 26,000 (spot D) stained blue. These components were present in all gels.

The overall patterns obtained with the nine serovars of *U. urealyticum* fell into two general groups; one group consisted of serovars II, IV, V, VII, VIII, and IX (group A), and the other consisted of serovars I, III, and VI (group B). Figure 2 shows representative gels from each group. Numbers were arbitrarily assigned to some of the spots. A number of spots appeared to be common to all strains examined (Table 1). These spots were similar not only in molecular weight and isoelectric point, but also in staining characteristics. For example, spots 14, 31 through 33, and 35 always stained yellow, and spots 28 and 29 were orange with a surrounding red halo. When the amount of 20% acrylamide in the gel was increased slightly, the differences between group A and group B strains were readily apparent, and the common cluster of

TABLE 1. Summary of prominent proteins in strains of *U. urealyticum* representing serovars I through IX

Group	Spot no.	Approx mol wt (10 ³)	Color	
Common ^a	1	9	Blue	
	7	15	Dark red-purple	
	14	20	Yellow	
	28	32	Red-orange	
	29	32	Red-orange	
	31	75	Yellow	
	32	100	Yellow	
	33	100	Yellow	
	35	140	Yellow	
Group A ^b	3	12	Red-orange	
	6	14.4	Blue-purple	
	9	18.5	Blue	
	10	19.5	Blue-orange	
	13	20	Red	
	15	21.5	Dark blue	
	16	21.5	Purple	
	17	21.5	Red	
	21	24.5	Dark blue	
	22	24.5	Purple	
	24	26	Maroon-black	
Group B ^c	4	12.2	Red-orange	
	5	13	Blue-purple	
	8	17.5	Maroon	
	11	19.5	Orange	
	12	20	Red	
	18	22	Red	
	19	22	Maroon-black	
	20	23	Blue	
	23	25	Red	
Specific ^d	II, III, IV, V, VI, VIII, IX	2	10.1	Blue-purple
	III, VI	25	28	Yellow-orange
	III, VI	26	28	Yellow-orange
	II, (IV?)	27	29	Red-purple
	IX	30	41	Red
	III, V, VI, VIII	34	130	Yellow

^a Found in all nine serovars.

^b Found in serovars II, IV, V, VII, VIII, and IX.

^c Found in serovars I, III, and VI.

^d Found only in the serovars listed.

proteins labeled 1 formed a consistently unique shape (Fig. 3).

Apparently unique spots were found in some of the strains examined. Serovar IX had a unique polypeptide that stained bright red and had a molecular weight of approximately 41,000 (spot 30) and an isoelectric point similar to that of medium component D. This spot was not found in any of the other strains. Spot 27 occurred consistently in the strain representing serovar II and was variable in serovar IV.

The sensitivity of silver-based staining procedures allows the direct examination of proteins from small samples without the need for radiola-

FIG. 2. Two-dimensional gel electrophoresis of two strains of *U. urealyticum*. Some prominent spots are numbered. (A) Serovar II, representing group A strains. (B) Serovar VI, representing group B strains. 200K, Molecular weight of 200,000.

beling. In addition, the staining procedure of Sammons et al. (12) produces protein spots with characteristic colors. This property facilitates visual analysis of gels and provides additional information on the characteristics of the proteins present. It has been suggested that some proteins may have different colors depending on posttranslational modification, amino acid con-

tent, the concentration of the protein in the gel, or the type of moiety that is covalently linked to the protein. Although it is not yet understood how the variety of colors are produced, it is clear that color is a unique and reproducible property of the proteins.

Deoxyribonucleic acid hybridization and gel electrophoresis have indicated clear differences

FIG. 3. Sections of two-dimensional gels of serovars I (A) and VII (B), showing low-molecular-weight proteins. The numbering system is the same as that used in Fig. 2.

between strains of *U. urealyticum*. Using different techniques, we were able to confirm the division of nine human *Ureaplasma* strains (representing serovars I through IX) into two major groups. Further work will be necessary to determine (i) whether there are more than two major electrophoretic groups within the species *U. urealyticum*, (ii) whether the electrophoretic patterns observed are related to the serological properties of the organisms, and (iii) whether there are biochemical differences between virulent and avirulent strains of *U. urealyticum*.

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