INTERNATIONAL JOURNAL OF SYSTEMATIC BACTERIOLOGY, Jan. 1980, p. 69-76
0020-7713/80/01-0069/08$02.00/0
Vol. 30, No. 1

Polyacrylamide Gel Electrophoresis of Whole-Cell Proteins of Porcine Strains of *Haemophilus*

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The protein patterns of sodium dodecyl sulfate-solubilized whole cells of porcine strains of *Haemophilus* were studied by using polyacrylamide gel electrophoresis. The pattern of *Haemophilus pleuropneumoniae* was very homogenous and was independent of the serological type. At least two different patterns could be distinguished in *Haemophilus parasuis*, suggesting some heterogeneity in this species. The results were highly reproducible and were not affected by growth conditions. Comparable patterns were obtained after solubilization with sodium taurocholate and sodium carbonate and after ultrasonic treatment, but not after phenol-acetic acid extraction. In addition, we compared porcine strains with human strains (*Haemophilus influenzae, Haemophilus parahaemolyticus*) and also with some bacteria of the genera *Pasteurella, Actinobacillus, Brucella, Moraxella,* and *Bordetella.* The species-specific picture is given mostly by the pattern of a group of proteins with molecular weights just above 68,000 (*Haemophilus*) and in the region of molecular weights between 23,000 and 40,000 and between 15,000 and 17,000 (*Haemophilus, Pasteurella, Actinobacillus*). These observations suggest the possible use of this method as an aid in studying the taxonomy of these bacteria.

The electrophoretic patterns obtained by polyacrylamide gel electrophoresis (PAGE) of cell proteins from bacteria has proved to be useful in taxonomic studies. Since these cell proteins are genetically directed, their patterns tend to express genetic relationships between microorganisms (29).

In recent years, reports have been published on the use of PAGE with a great variety of microorganisms, ranging from mycoplasmas (5, 6, 27-29, 40) to bacteria and even fungi (*Aspergillus fumigatus*) (38); the bacteria have included brucellae (20), staphylococci (3), enteric bacteria (26, 30, 32, 33, 44), streptomycetes (7), yersiniae (10), mycobacteria (8), streptococci (17, 18), campylobacters (vibrios) (21), neisseriae (24, 31, 45), *Corynebacterium diphtheriae* (16), *Erysipelothrix rhusiopathiae* (43), and *Pasteurella haemolytica* (42). For the genus *Haemophilus*, only a few studies of this type have been reported. Neumann and Hinz (22) found that the protein patterns of strains of *Haemophilus paragallinarum, Haemophilus avium, Haemophilus parainfluenzae,* and *Haemophilus parasuis* are quite different, even among strains within the same species, so that the method has been judged to be unreliable for taxonomic purposes.

It has been clearly demonstrated that the cytoplasmic membranes and whole-cell proteins of mycoplasmas and also of L-forms of bacteria show highly reproducible and species-specific electrophoretic patterns with PAGE (28, 39, 40). It is likely in such cases that PAGE can be used as a taxonomic tool. In the case of other bacteria, the majority of authors have observed similarities in the electrophoretic patterns within a genus (20, 32, 41) or within a species (7, 8, 10, 18, 20, 24, 31, 42) but not between genera (20, 32, 41).

Despite the rather complex structure of bacterial cells, the observations cited above tend to confirm the usefulness of PAGE in studying the genetic relationships of bacteria. However, a few incongruous results (17, 24, 43) cast some doubts on the prudence of accepting PAGE patterns of whole-cell proteins as taxonomically useful. These incongruities point out the need for standardizing the solubilization and electrophoretic methods, as well as the need for a better knowledge of the influence of growth conditions on bacterial cell composition. It has been shown (1, 11, 12, 34-37, 46) that bacterial cytoplasmic membranes and outer membrane proteins may have some influence on the protein patterns. The purpose of the study reported here was to determine the electrophoretic patterns of whole-cell proteins from *Haemophilus* strains of porcine origin and the relationship of these patterns to those of some human strains of *Haemophilus* and of organisms in other genera.

MATERIALS AND METHODS

Bacterial strains. The strains used in this study are listed in Table 1.

Bacterial suspensions. Bacteria were cultured on

TABLE 1. *List of strains used in this study*

Laboratory no.	Name	Strain and/or serovar designation	Source[a]	Lesion
	H. parasuis	A 9		?
	H. parasuis	B 26		?
	H. parasuis	C 5	1	?
	H. parasuis	D 74		?
1597	*H. parasuis*		2	Pneumonia (pig)
899	*H. parasuis*			Pneumonia (pig)
1163	*H. parasuis*			Glässer disease (pig)
1068	*H. parasuis*			Glässer disease (pig)
1500	*H. parasuis*			Glässer disease (pig)
1722	*H. parasuis*		2	Glässer disease (pig)
1598	*H. parasuis*			Glässer disease (pig)
1384	*H. parasuis*			Glässer disease (pig)
4074[b]	*H. pleuropneumoniae*	Serovar 1, ATCC 27088	2	Contagious pleuropneumonia (pig)
S 1535	*H. pleuropneumoniae*	Serovar 2, ATCC 27089	2	Contagious pleuropneumonia (pig)
S 1421	*H. pleuropneumoniae*	Serovar 3, ATCC 27090	2	Contagious pleuropneumonia (pig)
M 62	*H. pleuropneumoniae*	Serovar 4	3	Contagious pleuropneumonia (pig)
K 17	*H. pleuropneumoniae*	Serovar 5	3	Arthritis (lamb)
2538/4	*H. pleuropneumoniae*	Serovar 1	Australia (4)	Contagious pleuropneumonia (pig)
S 447	*H. pleuropneumoniae*	Serovar 2	Switzerland (2)	Contagious pleuropneumonia (pig)
HP 8	*H. pleuropneumoniae*	Serovar 2	Japan (4)	Contagious pleuropneumonia (pig)
S 43	*H. pleuropneumoniae*	Serovar 3	Switzerland (2)	Contagious pleuropneumonia (pig)
12960	*H. pleuropneumoniae*	Serovar 5	Holland (4)	Contagious pleuropneumonia (pig)
95905	*H. pleuropneumoniae*	Serovar 5	Taiwan (4)	Contagious pleuropneumonia (pig)
266-1970HAE	*H. pleuropneumoniae*	Serovar 5	Belgium (4)	Contagious pleuropneumonia (pig)
Greenfield	*H. pleuropneumoniae*	Serovar 5	Canada (4)	Contagious pleuropneumonia (pig)
L 349	*Haemophilus* sp.	Minor group	4	Pneumonia (pig)
LW 202	*Haemophilus* sp.	Minor group	4	Pneumonia (pig)
	H. influenzae	b (51)	5	? (Human)
	H. parahaemolyticus	536	6	? (Human)
S 847	*Pasteurella multocida*	Type A	2	Pneumonia (pig)
W 241	*Pasteurella haemolytica*		2	Pneumonia (sheep)
W 702	*Actinobacillus lignieresii*		2	Abscess (cattle)
S 302	*Actinobacillus lignieresii* (hemolytic variant)		2	Pneumonia (pig)
	Brucella abortus	W 99	7	? (Cattle)
	Moraxella bovis	NCTC 9426	8	? (Cattle)
S 485	*Bordetella bronchiseptica*		2	Atrophic rhinitis (pig)

[a] Sources: 1, K. Bakos, Veterinärmedicinska Anstalt, Stockholm, Sweden; 2, our own isolates; 3, E. L. Biberstein, University of California, Davis; 4, source as mentioned in Kilian et al. (15); 5, T. Omland, University of Oslo, Oslo, Norway; 6, M. Pittman, National Institutes of Health, Bethesda, Md.; 7, Central Veterinary Laboratory, Weybridge, England; 8, S. D. Henriksen, University of Oslo.

[b] Proposed neotype strain (15).

chocolate agar (10% sheep blood in blood agar base [Oxoid] heated at 80°C for 10 min in two steps). For comparative purposes we also used brain heart infusion agar (Difco Laboratories) enriched with 1.25% yeast extract (Fleishman active dry yeast) and Trypticase soy agar (BBL Microbiology Systems) mixed with 10 μg of nicotinamide adenine dinucleotide (Sigma Chemical Co.) per ml (NAD agar). The cells were harvested in saline and washed three times in saline.

Protein determination. The protein content of the bacteria was determined by the Hartree (9) modification of the Lowry method.

Preparation of bacterial extracts. (i) SDS solubilization. Washed bacteria were suspended in 0.0125 M phosphate buffer (pH 7.4) containing 1% sodium dodecyl sulfate (SDS) and 1% β-mercaptoethanol at a protein concentration of 1 mg/ml, and the suspension was heated to boiling for 3 min. Complete solubilization occurred. For comparison, solubilization was also done at 60°C for 1 h and at 37°C overnight.

(ii) Sodium taurocholate solubilization. Sodium taurocholate solubilization was performed by the method described by Omland (25). Washed bacteria were suspended in 0.005 M phosphate buffer (pH 7.4) containing 0.005 M NaCl to give a concentration of 1 mg of cell protein per ml. To this suspension was added 0.1 volume of a 10% solution of sodium taurocholate (prepared in distilled water). This mixture was incubated for 1 h in a water bath at 37°C and was centrifuged at 48,000 × *g* for 45 min. The supernatant was mixed with an equal volume of phosphate buffer containing 1% SDS.

(iii) Sodium carbonate solubilization. Sodium carbonate solubilization was performed by the method described by Omland (25). A suspension of 1 mg of cell protein per ml was treated with 1% sodium carbonate for 1 h in a water bath at 37°C. The pH was then lowered to 8.0 (from about 10.0) by adding 1 M acetic acid. The suspension was then centrifuged at 48,000 × *g* for 45 min.

(iv) Ultrasonic disintegration. Washed bacteria were suspended in 0.01 M phosphate buffer (pH 7.4) containing 0.14 M NaCl to give a concentration of 2 mg of cell protein per ml. This suspension was treated with a Branson Sonifier at full output for 3 min; the sample was maintained at −30°C (methanol-water mixture with dry ice) to prevent overheating. The clear fluid of disrupted cells was mixed with an equal volume of phosphate buffer containing 1% SDS and 1% β-mercaptoethanol.

Phenol-acetic-water extract. Phenol-acetic-water extract was performed as described by Razin and Rottem (29).

PAGE. For PAGE, the phosphate-buffered, 7.5% gel system described by Morowitz and Terry (19) was used; 1% SDS was added to the system, and the flat gel slab method, employing equipment from Biowerk (Biozentrum, Basel), was used. Samples (20 to 40 μl) of cell protein (1 mg/ml) were layered with 30% glycerol and subjected to electrophoresis at 100 mA for 3 h.

The slabs were stained for proteins with Coomassie brilliant blue R (4). Alternatively, for phenol-acetic acid-water extracts, a gel system containing 5 M urea (29) was used. For molecular weight standards, a mixture of the following reference substances was used: bovine serum albumin (molecular weight 68,000; Fluka, Buchs, Switzerland); catalase (60,000; Sigma); ovalbumin (43,000; Serva, Heidelberg, West Germany); pepsin (35,000; Sigma); trypsin (23,300; Sigma); myoglobin (17,200; Sigma); and hemoglobin (15,500; Sigma).

RESULTS

Electrophoretic patterns of porcine strains of *Haemophilus*. The protein patterns obtained with SDS-solubilized whole cells of the different *Haemophilus* strains of porcine origin included in this study are shown in Fig. 1. The interpretation of a specific pattern was based on R_f values rather than on the intensity of the bands. We tentatively present in Fig. 1 a diagram of the overall patterns of *H. parasuis* and *Haemophilus pleuropneumoniae*. The most critical differences between the species of *H. parasuis* and *H. pleuropneumoniae* are in the patterns of groups of bands with molecular weights just above 68,000 and between 23,000 and 40,000.

Within *H. parasuis*, there is a good deal of similarity among the major proteins of strains A, C, and D. However, quite a different pattern is evident for strain B, especially in the R_f value of a band of a molecular weight of about 37,000.

For the three serovars of *H. pleuropneumoniae*, the overall patterns are identical.

For the *Haemophilus* strains of the "minor group," two different patterns were obtained, suggesting heterogeneity within this group; strain L 349 seems to be closely related to *H. pleuropneumoniae*.

Effect of growth conditions. (i) Growth medium. With the growth from chocolate agar, brain heart infusion agar, and NAD agar, no differences in the major protein patterns of SDS-solubilized washed cell suspensions of *H. pleuropneumoniae* strains were detected.

(ii) Age of culture. Preliminary investigations with *H. parasuis* strain A and *H. pleuropneumoniae* showed that the length of incubation on chocolate agar had no marked influence on the overall cell protein pattern, even after 120 h of incubation. We observed, however, that in very young cultures (5 h) the quantity of high-molecular-weight proteins (molecular weights over 68,000) was less than in older cultures.

(iii) Fresh isolates and subcultures. The overall patterns of the major proteins of fresh isolates of *H. pleuropneumoniae* and *H. parasuis* did not differ significantly from those of the collection strains. Also, the use of subcultures had no effect on the patterns.

Effect of extraction procedure. The effects of classical solubilizing agents (SDS, sodium taurocholate, sodium carbonate, phenol-acetic acid extraction) and ultrasonic treatment on the sol-

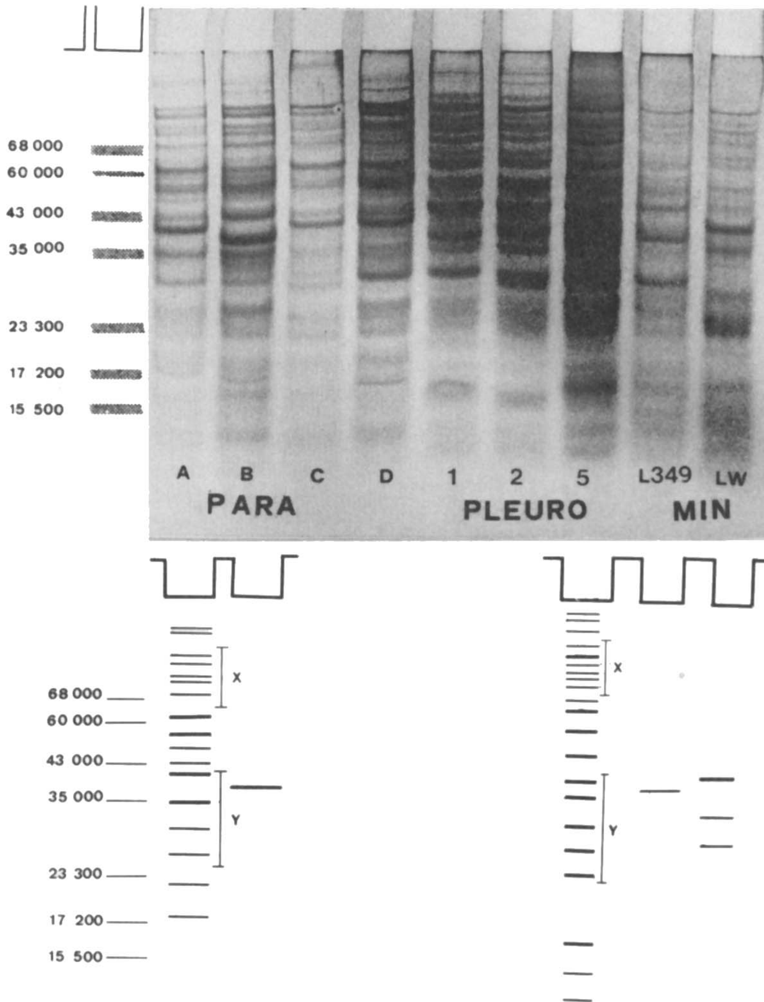

FIG. 1. *PAGE of various SDS-solubilized porcine strains. PARA, H. parasuis types A, B, C, and D; PLEURO, H. pleuropneumoniae serovars 1, 2, and 5; MIN, Haemophilus sp. minor group. Below the gels are diagrams of the patterns of H. parasuis type A and H. pleuropneumoniae with the species-specific groups of bands (X and Y) in addition to the critical bands of H. parasuis type B and Haemophilus sp. minor group. Numbers on the left are molecular weights.*

ubilization of cells of *H. pleuropneumoniae* serovar 2 were compared in order to determine what effect, if any, the extraction procedure had on the protein patterns. Neither the different solubilization procedures nor the ultrasonic treatment had any influence on the protein pattern. However, SDS treatment provided a remarkably clear pattern, suggesting a more effective solubilization. On the other hand, extracts obtained after treatment with phenol-acetic acid could not be investigated with the PAGE system which we used because the loaded proteins migrated in the opposite direction. Inverting the electrodes produced a picture of very poor quality, one not comparable at all with the pictures obtained with the other extracts. Pictures obtained with the gel system containing 5 M urea were inferior to those obtained with the SDS gel system.

We also investigated the possible effects of temperature and time of action of SDS on the solubilization procedure (boiling for 3 min; 60°C for 1 h, and 37°C overnight). Short boiling (3 min) offered the best representation of the high-molecular-weight proteins.

Electrophoretic patterns of diagnostic

strains. **(i) *H. pleuropneumoniae*.** Figure 2 shows the electrophoretic patterns of eight strains of *H. pleuropneumoniae;* these strains were selected at random, but differences in serovar and geographic distribution were taken into consideration. The patterns were similar for all strains.

(ii) *H. parasuis*. Two strains (1597 and 899) freshly isolated from cases of pneumonia and six strains from Glässer disease were used. Figure 3 shows the electrophoretic patterns which distinguish between respiratory strains (strains 1597 and 899; related to the patterns of *H. parasuis* strains A, C, and D [critical bands in Fig. 1]) and Glässer strains (strains 1163, 1500, 1722, 1598, and 1384; related to *H. parasuis* strain B). The pattern of strain 1068 (Glässer), although having some similarities to the patterns of the respiratory strains, seems to be unrelated to the pattern of any of the species.

Comparison of the protein patterns of *Haemophilus* species with those of other genera. Figure 4 shows a comparison of the

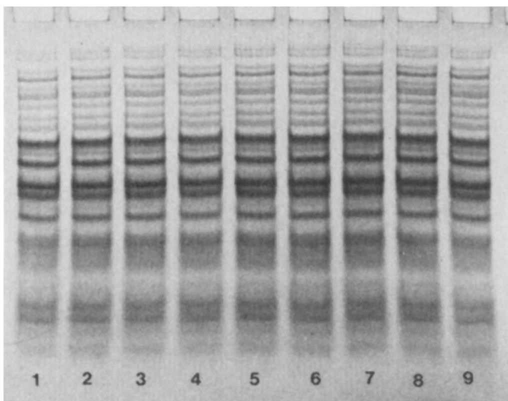

FIG. 2. *PAGE of SDS-solubilized isolates of H. pleuropneumoniae from various countries. Gel 1, Serovar 1, (4074) from Argentina (proposed neotype); gel 2, serovar 1 from Australia; gel 3, serovar 2 from Switzerland; gel 4, serovar 2 from Japan; gel 5, serovar 3 from Switzerland; gel 6, serovar 5 from Holland; gel 7, serovar 5 from Taiwan; gel 8, serovar 5 from Belgium; gel 9, serovar 5 from Canada.*

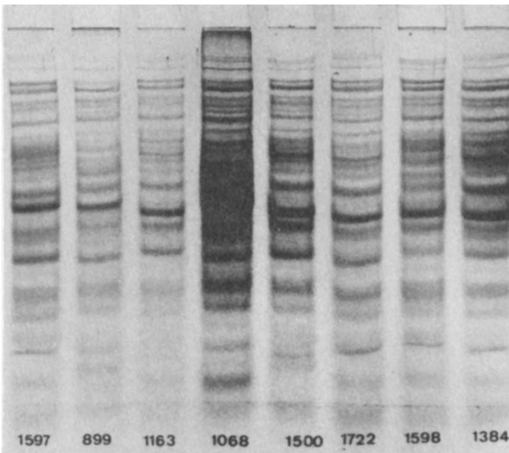

FIG. 3. *PAGE of SDS-solubilized fresh isolates of H. parasuis.*

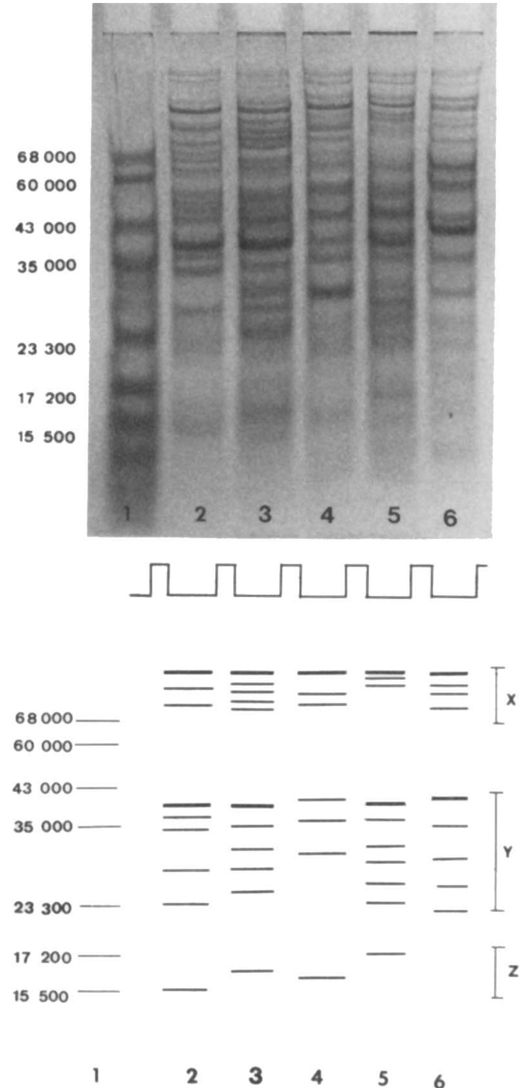

FIG. 4. *Protein patterns of some Haemophilus strains. Gel 1, Molecular weight standard; gel 2, H. influenzae b strain 51; gel 3, H. parahaemolyticus strain 536; gel 4, H. pleuropneumoniae strain S 1536; gel 5, Haemophilus sp. minor group strain 202; gel 6, H. parasuis strain A9. Below the gels are diagrams of the species-specific groups of bands (X, Y, and Z). Numbers on the left are molecular weights.*

protein patterns of *H. influenzae* b, *H. parahaemolyticus*, *Haemophilus* strains of the minor group, and *H. parasuis*. The main differences in the patterns occur in the regions of molecular weights just above 68,000, between about 23,000 and 40,000, and between 15,000 and 17,000.

In Fig. 5 strains of some *Haemophilus* species are compared with strains of *Pasteurella*, *Actinobacillus*, *Brucella*, *Moraxella*, and *Bordetella*. As within the genus *Haemophilus*, there is a similarity in the patterns of the more cathode-located proteins of strains of *Pasteurella* and *Actinobacillus*. However, this was not the case with strains of *Brucella*, *Moraxella*, and *Bordetella*. Despite the relative similarity in the patterns of some of the strains, the general protein pattern was quite specific for each strain examined. It is interesting to note that there are distinct pattern differences between *Pasteurella multocida* and *P. haemolytica* and between *Actinobacillus lignieresii* and its hemolytic variant, which in turn differs from *P. haemolytica*.

DISCUSSION

This study of the protein patterns of cells of porcine strains of *Haemophilus* shows that un-

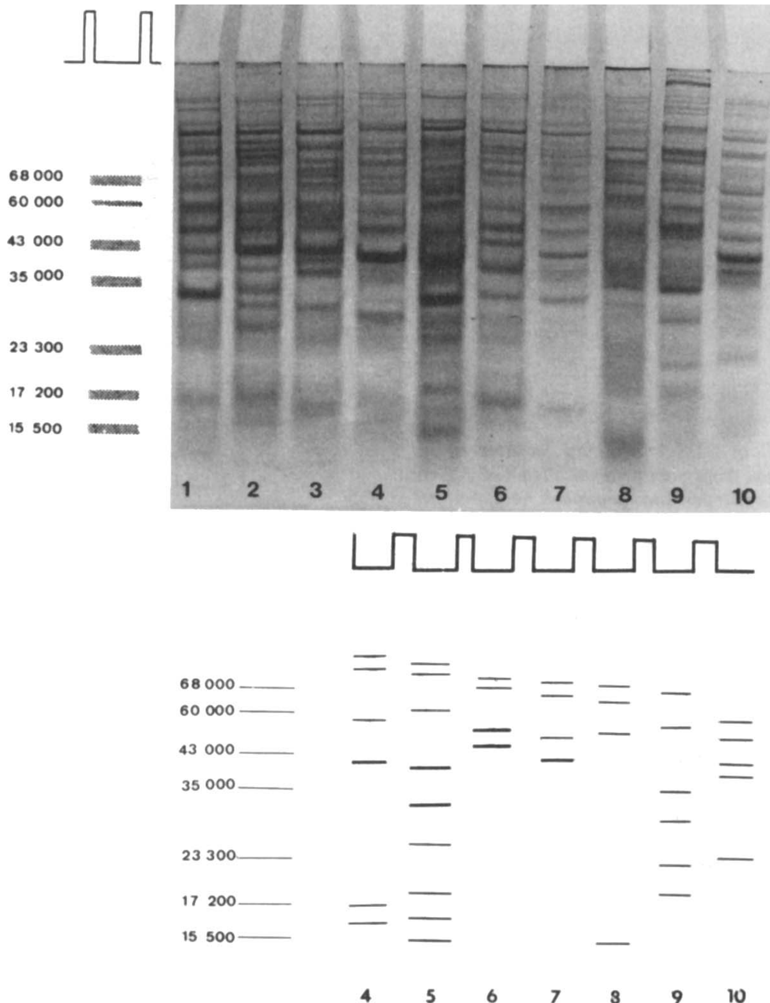

FIG. 5. *Protein patterns of SDS-solubilized cells of some haemophili and other bacteria. Gel 1, H. pleuropneumoniae strain S 1536; gel 2, H. parahaemolyticus strain 536; gel 3, H. influenzae b; gel 4, Pasteurella multocida; gel 5, Pasteurella haemolytica; gel 6, Actinobacillus lignieresii (hemolytic); gel 7, Actinobacillus lignieresii; gel 8, Brucella abortus strain W 99; gel 9, Moraxella bovis; gel 10, Bordetella bronchiseptica. Below the gels are diagrams of species-specific patterns for the genera Pasteurella (lanes 4 and 5), Actinobacillus (lanes 6 and 7), Brucella (lane 8), Moraxella (lane 9), and Bordetella (lane 10). Numbers on the left are molecular weights.*

der standard conditions, a PAGE system employing SDS gels can provide highly reproducible results. Furthermore, the protein patterns are species specific, and the overall patterns are influenced neither by the growth conditions (type of medium, age of the culture, subcultures, etc.) nor by the methods of solubilizing the cells, except for treatment with phenol-acetic acid. This treatment seems to produce conditions of cell solubilization which are less than optimal for PAGE, explaining perhaps some of the misleading results obtained with *H. paragallinarum* and *H. avium* (22).

It is characteristic of *Haemophilus* cells to be solubilized easily by chemical agents, such as sodium taurocholate and sodium carbonate (25). Detergents, such as SDS, are also very efficient. This characteristic leads to a rapid and complete solubilization of cell proteins, which allows a high degree of reproduction of the electrophoretic pattern.

The electrophoretic pattern of the cell proteins of *H. pleuropneumoniae* is very specific and is independent of serological type. This is not surprising since serological specificity is due to capsular polysaccharides (23), which are not involved in protein determinations by PAGE. It is to be noted that at least one of the two strains of the minor group of haemophili shows a pattern definitely distinct from that of *H. pleuropneumoniae*, confirming the results of the study by Kilian et al. (15). The fact that the pattern of *H. pleuropneumoniae* is very different from that of *H. parahaemolyticus* is a further confirmation of the distinctiveness of the two species (14, 15).

The protein pattern of Bakos strains A, B, C, and D of *H. parasuis* (2; K. Bakos, Ph.D. thesis, Veterinar medicinska, Anstalt, Stockholm, 1955) permits the distinction of at least two separate patterns, that of strains A, C, and D on the one hand and that of strain B on the other. An atypical pattern was obtained from the field strains, suggesting a third group. These observations indicate a certain heterogeneity among *H. parasuis* strains. This is not surprising since *H. parasuis* has been inadequately investigated. The pattern obtained with the small number of strains studied suggests that strains isolated from Glässer syndrome and associated with pattern B are quite homogeneous and different from the respiratory strains, which are associated with the pattern of strains A, C, and D. This observation and the exact status of the *H. parasuis* groups have to be examined further in studies employing more field strains and the newly established neotype strains of the various *Haemophilus* species.

Numerical taxonomy studies have shown that haemophili are closely related to pasteurellae and actinobacilli but not to brucellae, moraxellae, and bordetellae (13). The results obtained here by PAGE of the cell proteins of a few strains seem to agree with the results obtained by numerical taxonomy and demonstrate the feasibility of using this technique in taxonomic studies of these bacteria.

In summary, this investigation of the protein patterns of porcine strains of *Haemophilus* shows the great possibilities which PAGE offers for studying the relationships of various bacteria, provided that standard conditions are used. Our aim was not to draw taxonomic conclusions but to determine the potential use of PAGE in taxonomic studies of the haemophili. A comprehensive taxonomic study would have to be done more systematically and would have to involve the customary phenetic and genetic criteria.

REPRINT REQUESTS

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LITERATURE CITED

1. **Ames, G. F.-L.** 1974. Resolution of bacterial proteins by polyacrylamide gel electrophoresis on slabs. J. Biol. Chem. **249**:634-644.
2. **Bakos, K., A. Nilsson, and E. Thal.** 1952. Untersuchungen über *Haemophilus suis*. Nord. Veterinaer Med. **4**: 241-255.
3. **Brückler, J., and H. Blobel.** 1970. Vertikale Polyacrylamid-Gel Disk-Elektrophorese von Staphylokokken-Proteinen. Zentralbl. Bakteriol. Parasitenkd. Infektionskr. Hyg. Abt. 1 Orig. **213**:161-169.
4. **Chrambach, A., R. A. Reisfeld, M. Wickoff, and J. Zaccari.** 1967. A procedure for rapid and sensitive staining of protein fractionated by polyacrylamide gel electrophoresis. Anal. Biochem. **20**:150-154.
5. **Daniels, M. J., and B. M. Meddins.** 1973. Polyacrylamide gel electrophoresis of mycoplasma proteins in sodium dodecyl sulphate. J. Gen. Microbiol. **76**:239-242.
6. **Dellinger, J. D., and D. E. Jasper.** 1972. Polyacrylamide gel electrophoresis of cell proteins of *Mycoplasma* isolated from cattle and horses. Am. J. Vet. Res. **33**:769-775.
7. **Gottlieb, D., and P. M. Hepden.** 1966. The electrophoretic movement of proteins from various streptomyces species as a taxonomic criterion. J. Gen. Microbiol. **44**: 95-104.
8. **Haas, H., Y. Davidson, and T. Sacks.** 1972. Taxonomy of mycobacteria studied by polyacrylamide gel electrophoresis of cell proteins. J. Med. Microbiol. **5**:51-37.
9. **Hartree, E. F.** 1972. Determination of protein: a modification of the Lowry method that gives a linear photometric response. Anal. Biochem. **48**:422-427.
10. **Hudson, B. W., and T. J. Quan.** 1975. Electrophoretic studies of the yersiniae. Am. J. Trop. Med. Hyg. **24**: 968-973.
11. **Inouye, M., and A. B. Pardee.** 1970. Changes of membrane proteins and their relation to deoxyribonucleic acid synthesis and cell division of *Escherichia coli*. J. Biol. Chem. **21**:5813-5819.
12. **Inouye, M., and M. Yee.** 1973. Homogeneity of envelope proteins of *Escherichia coli* separated by gel electrophoresis in sodium dodecyl sulfate. J. Bacteriol. **113**: 304-312.
13. **Johnson, R., and P. H. A. Sneath.** 1973. Taxonomy of

Bordetella and related organisms of the families *Achromobacteriaceae*, *Brucellaceae*, and *Neisseriaceae*. Int. J. Syst. Bacteriol. **23**:381-404.

14. **Kilian, M.** 1976. A taxonomic study of the genus *Haemophilus* with the proposal of a new species. J. Gen. Microbiol. **93**:9-62.
15. **Kilian, M., J. Nicolet, and E. L. Biberstein.** 1978. Biochemical and serological characterization of *Haemophilus pleuropneumoniae* (Matthews and Pattison, 1961) Shope 1964 and proposal of a neotype strain. Int. J. Syst. Bacteriol. **28**:20-26.
16. **Larsen, S. A., S. T. Bickham, T. M. Buchanan, and W. T. Jones.** 1971. Polyacrylamide gel electrophoresis of *Corynebacterium diphtheriae*: a possible epidemiological aid. Appl. Microbiol. **22**:885-890.
17. **Larsen, S. A., C. D. Webb, and M. D. Moody.** 1969. Acrylamide gel electrophoresis of group A streptococcal cell walls. Appl. Microbiol. **17**:31-33.
18. **Lund, B. M.** 1965. A comparison by the use of gel electrophoresis of soluble protein components and esterase enzymes of some group D streptococci. J. Gen. Microbiol. **40**:413-419.
19. **Morowitz, H. J., and T. M. Terry.** 1965. Characterization of the plasma protein membrane of *Mycoplasma laidlawii*. Biochim. Biophys. Acta **183**:276-294.
20. **Morris, J. A.** 1973. The use of polyacrylamide gel electrophoresis in taxonomy of *Brucella*. J. Gen. Microbiol. **76**:231-237.
21. **Morris, J. A., and R. W. A. Park.** 1973. A comparison using gel electrophoresis of cell proteins on campylobacteria (vibrios) associated with infertility, abortion and swine dysentery. J. Gen. Microbiol. **78**:165-178.
22. **Neumann, U., and K. H. Hinz.** 1977. Elektrophoretische Auftrennung von *Haemophilus*-Proteinen im Polyacrylamid-Gel. Zentralbl. Bakteriol. Parasitenkd. Infektionskr. Hyg. Abt. 1 Orig. Reihe A **238**:244-250.
23. **Nicolet, J.** 1971. Sur l'hémophilose du porc. III. Différenciation sérologique de *Haemophilus parahaemolyticus*. Zentralbl. Bakteriol. Parasitenkd. Infektionskr. Hyg. Abt. 1 Orig. Reihe A **216**:487-495.
24. **Noble, R. C., and S. C. Schell.** 1978. Acrylamide gel electrophoresis of proteins of *Neisseria gonorrhoeae* as an epidemiological tool. Infect. Immun. **19**:178-186.
25. **Omland, T.** 1964. Serological studies on *Haemophilus influenzae* and related species. Acta Pathol. Microbiol. Scand. **62**:79-88.
26. **Osborn, M. J., J. E. Gander, E. Parisi, and J. Carson.** 1972. Mechanism of assembly of the outer membrane of *Salmonella typhimurium*. J. Biol. Chem. **247**:3962-3972.
27. **Paroz, P., M. Krawinkler, and J. Nicolet.** 1977. Verwendung der Polyacrylamid-Gel-Elektrophorese in der Diagnostik von Nutztier-Mykoplasmen. Zentralbl. Veterinaermed. Reihe B **24**:668-677.
28. **Razin, S.** 1968. *Mycoplasma* taxonomy studied by electrophoresis of cell proteins. J. Bacteriol. **96**:687-694.
29. **Razin, S., and S. Rottem.** 1967. Identification of *Mycoplasma* and other microorganisms by polyacrylamide gel electrophoresis of cell proteins. J. Bacteriol. **94**:1807-1810.
30. **Rosenbusch, J. P.** 1974. Characterization of the major envelope protein of *Escherichia coli*. J. Biol. Chem. **249**:8019-8029.
31. **Russell, R. R. R., K. G. Johnson, and I. J. McDonald.** 1975. Envelope proteins of *Neisseria*. Can. J. Microbiol. **21**:1519-1534.
32. **Sacks, T. G., H. Haas, and S. Razin.** 1969. Polyacrylamide gel electrophoresis of cell proteins of *Enterobacteriaceae*. Isr. J. Med. Sci. **5**:49-55.
33. **Schnaitman, C. A.** 1970. Protein composition of the cell wall and cytoplasmic membrane of *Escherichia coli*. J. Bacteriol. **104**:890-901.
34. **Schnaitman, C. A.** 1973. Outer membrane proteins of *Escherichia coli*. I. Effect of preparative conditions on the migration of protein in polyacrylamide gels. Arch. Biochem. Biophys. **157**:541-552.
35. **Schnaitman, C. A.** 1973. Outer membrane proteins of *Escherichia coli*. II. Heterogeneity of major outer membrane polypeptides. Arch. Biochem. Biophys. **157**:553-560.
36. **Schnaitman, C. A.** 1974. Outer membrane proteins of *Escherichia coli*. III. Evidence that the major protein of *Escherichia coli* 0111 outer membrane consists of four distinct polypeptide species. J. Bacteriol. **118**:442-454.
37. **Schnaitman, C. A.** 1974. Outer membrane proteins of *Escherichia coli*. IV. Differences in outer membrane proteins due to strain and cultural differences. J. Bacteriol. **118**:454-465.
38. **Sorenson, W. G., H. W. Larsh, and S. Hamp.** 1971. Acrylamide gel electrophoresis of proteins from *Aspergillus* species. Am. J. Bot. **58**:588-593.
39. **Theodore, S. T., J. R. King, and R. M. Cole.** 1969. Identification of L-forms by polyacrylamide gel electrophoresis. J. Bacteriol. **97**:495-499.
40. **Theodore, S. T., J. G. Tully, and R. M. Cole.** 1971. Polyacrylamide gel identification of bacterial L-forms and *Mycoplasma* species of human origin. Appl. Microbiol. **21**:272-277.
41. **Theodore, S. T., J. R. King, J. G. Tully, and R. M. Cole.** 1970. Polyacrylamide gel patterns of microorganisms. Dev. Ind. Microbiol. **11**:122-138.
42. **Thompson, D. A., and D. L. Mould.** 1975. Protein electrophoretic pattern of *Pasteurella haemolytica*. Res. Vet. Sci. **18**:342-343.
43. **White, T. G., and F. K. Mirikitani.** 1976. Some biological and physical-chemical properties of *Erysipelothrix rhusiopathiae*. Cornell Vet. **66**:152-163.
44. **Wu, M. C., and E. C. Heath.** 1973. Isolation and characterization of lipopolysaccharide protein from *Escherichia coli*. Proc. Natl. Acad. Sci. U.S.A. **70**:2572-2576.
45. **Zollinger, W. D., D. L. Kasper, B. J. Vettri, and M. S. Artenstein.** 1972. Isolation and characterization of a native cell wall complex from *Neisseria meningitidis*. Infect. Immun. **6**:835-851.
46. **Zoon, K. C., and J. J. Scocca.** 1975. Constitution of the cell envelope of *Haemophilus influenzae* in relation to competence for genetic transformation. J. Bacteriol. **123**:666-677.