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A New Phage Typing Scheme for *Proteus mirabilis* and *Proteus vulgaris* Strains

1. Morphological Analysis

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ABSTRACT. A new bacteriophage typing set, composed of 22 phages, was established as a tool for differentiation of *Proteus* strains. All the phages were tailed and included 4 morphological types (A1, A2, B1 and C1). They were classified into the families *Myoviridae*, *Siphoviridae* and *Podoviridae*. From the set, 19 phages had double-stranded DNA and 3 were single-stranded DNA phages.

The first attempts to use bacteriophage typing of *Proteus mirabilis* and *P. vulgaris* strains were made 10 years ago to improve the identification and differentiation of clinical *Proteus* isolates. Several phage-typing systems have been set up but none of them has been accepted as an international standard (cf. Rishe 1973). In our early experiments, we used the most convenient set, the S-J phage set, developed at that time by W.C. Schmidt, consisting of 13 specific *Proteus* phages (Schmidt and Jeffries 1974). However, neither the cultivation of *Proteus* isolates on EDNA medium, as recommended by the authors, nor the results of typing were satisfactory. Later, we adopted the approach of Hickman and Farmer (1976), who also employed the S-J phage set but with a modified method. However, about 20 % of our *Proteus* isolates were sensitive to some phages of the S-J set (Sekaninová 1984). This might have been due to the fact that our *Proteus* strains were isolated in geographical conditions different from those in which the original S-J set phages were obtained.

Therefore we isolated a group of *Proteus* bacteriophages from local sources with the intention to develop a new, more suitable bacteriophage typing scheme.

MATERIALS AND METHODS

Bacterial strains. Isolation and propagation of bacteriophages were carried out with 177 strains of *Proteus mirabilis* isolated from the clinical material provided by the *Municipal Health Service* in Brno, 478 isolates of *P. mirabilis* and 15 strains of *P. vulgaris* from urine samples collected at the *Department of Urology of the Teaching Hospital* in Brno and 9 strains of *P. mirabilis* (propagating strains for the S-J set phages) obtained from Dr. W.C. Schmidt (*Department of Microbiology, Wayne State University, School of Medicine*, Detroit, Michigan, USA).

Isolation of bacteriophages. Samples of raw sewage effluents were collected at the Brno sewage treatment plant and the Brno poultry abattoir between September 1980 to January 1987. Daily amounts of 100 mL collected on 5 consecutive days were pooled to make a sample for isolation. Isolation, purification and propagation procedures for bacteriophages were based on the methods described by Adams (1959), Bradley (1967) and Hickman and Farmer (1976). All of the phages isolated were purified by a repeated single-plaque isolation procedure until they formed homogeneous plaques on the propagating strains.

Media. Isolation, cultivation and biochemical identification of *Proteus* strains were carried out according to the procedures described by Schindler *et al.* (1979) with the addition of Brolacin Agar (*Merck*, Darmstadt). Bacteriophages were isolated, propagated and maintained using Tryptone Soya

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Broth, Brain Heart Infusion, Agar (all *Oxoid*) and Agar-Noble (*Difco*).

Electron microscopy. Lysates (PFU titre 1/pL, *i.e.* 10^9/mL) were centrifuged at 10 000 *g* for 20 min. Purified phages were deposited on copper grids provided with carbon-coated Formvar films and stained with 2 % potassium phosphotungstate or 0.5 % ammonium molybdate.

In all the phages, the diameter of the head was measured and, in the tailed phages, the total length of the tail was assessed. In contractile tails, the length recorded was that of the non-contracted tail. In phages with elongated heads, the dimensions along both long and short axes were measured. These measurements provided a basis for classification of the selected phages into morphotypes, as suggested by Bradley (1967) and Ackermann and Eisenstark (from Ackermann and DuBow 1987*a*) and into genera according to an international classification (Francki *et al.* 1991).

Isolation of phage DNA. Phage lysates were centrifuged at a low speed before the isolation of phage nucleic acid. A solution of 20 % polyethylene glycol in 2.5 mol/L NaCl was added to each purified lysate in an amount equal to 1/10 of the volume of the lysate. After 30 min of incubation at room temperature, the lysate was centrifuged at a frequency of 415 Hz (rotor, Beckman SW 28) for 30 min. The sediment was resuspended in 600 μL TE buffer (10 mmol/L Tris, pH 7.5, 1 mmol/L EDTA). Proteinase K and sodium dodecyl sulfate were added to the pure concentrated phage lysate to give final concentrations of 0.5 g/L and 1 %, respectively. After 1 h of incubation at 37 °C, the nucleic acid was purified by extraction with phenol, phenol–chloroform and chloroform. Ethanol-precipitated nucleic acid was resuspended in 100 μL TE buffer (Maniatis *et al.* 1982).

Determination of nucleic acid type. One μg of nucleic acid was incubated with 2 U of S1 nuclease, 0.5 g/L of DNAase I or 0.5 g/L of RNAase at 37 °C for 30 min. Resistance or sensitivity to these enzymes was analyzed by electrophoresis in 0.7 % agarose gel.

Restriction enzyme analysis and molecular size determination. Five μL of DNA (approx. 1 μg) was digested with restriction endonuclease *Hin*dIII (*New England Biolabs*) and separated by electrophoresis in 0.7 % agarose gel. After staining with ethidium bromide, the total molecular size of phage DNA was determined as a total of the molecular sizes of all restriction fragments using the λ/*Hin*dIII DNA as a molecular size standard.

RESULTS

Thirty seven *Proteus* phages were isolated and 22 of them were selected to compose a new typing set; the phages were designated **1**, **2**, **4**, **5**, **6**, **7**, **9B**, **10A**, **13**, **16**, **17**, **19A**, **20**, **22**, **23**, **24**, **25**, **26**, **27**, **30**, **32A**, and **33**. Phages **13** and **17** were obtained by spontaneous lysis of clinical strains of *P. mirabilis* isolated from the urinary tracts of patients at the urological ward. According to their morphology determined by electron microscopy, the phage particles were classified into 4 morphotypes and 3 families according to Bradley (1967) and Ackermann and Eisenstark (from Ackermann and DuBow 1987*a*). Fifty nine % (13 out of 22) of the phages had hexagonal heads and short non-contractile tails (family *Podoviridae*), 7 phages had hexagonal heads and long contractile tails (family *Myoviridae*) and the remaining 3 phages with hexagonal heads had long, non-contractile tails (family *Siphoviridae*). The dimensions of phages of the *Siphoviridae* and *Podoviridae* families were similar and these phages had morphotypes B1 and C1, respectively (Fig. 1c–e). The dimensions of the phages belonging to the family *Myoviridae* were different. Five phages had morphotype A1 and two had morphotype A2 (Table I, Fig. 1a,b).

The families *Myoviridae* and *Siphoviridae* produced mostly very small or small plaques (Fig. 1a–c) and the family *Podoviridae* produced medium-sized or large plaques usually surrounded by irregular zones, some with small circular depressions near the plaque centre (Fig. 1d,e).

Restriction endonuclease *Hin*dIII digested the DNA in 19 out of 22 *Proteus* typing set phages (Fig. 2). The sum of the molecular sizes of all DNA fragments in each analyzed phage gave a value which ranged between 40 and 50 kb, with the exception of phage **13**/807 (molecular size approx. 62 kb) and phage **24**/860 (approx. 100 kb). The nucleic acid from 3 *Proteus* phages (**22**/608, **30**/860 and 5/742), however, failed to be digested by the repeated procedures of purification and isolation or by treatment with a different restriction endonuclease (*Bam*HI). The nucleic acids of these 3 phages were resistant to the activity of RNAase and sensitive to the activity of DNAase and S1 nuclease.

Table I. Morphotypes, nucleic acid contents and main dimensions of *Proteus* phages

Phage	MTP[a]	dsDNA size, kb	Diameter of head, nm[b]	Length of tail, nm[b]	
				non-contractile	contractile
1/1004	B1	45.2	90.0	145.0	-
2/44	A1	49.6	61.1	-	84.1
4/545	A1	42.2	62.8	-	51.5
5/742	B1	ssDNA	104.0	168.0	-
6/1004	A1	45.2	65.7	-	72.1
7/549	C1	42.5	69.4	17.5	-
9B/2	C1	42.5	65.4	35.6	-
10A/31	C1	42.5	67.2	40.8	-
13/807	A1	62.2	74.0	-	54.0
16/789	C1	42.5	63.8	20.9	-
17/971	C1	45.4	68.1	34.0	-
19A/653	C1	38.0	63.1	22.9	-
20/826	A1	40.5	64.4	-	68.4
22/608	A2	ssDNA	87.3-107.7	-	111.3
23/532	C1	47.3	62.1	27.6	-
24/860	B1	99.0	86.9	182.1	-
25/909	C1	40.6	63.3	31.7	-
26/219	C1	42.5	62.3	32.2	-
27/953	C1	55.7	69.8	19.0	-
30/860	A2	ssDNA	86.7-101.4	-	106.7
32A/909	C1	40.5	70.4	18.2	-
33/971	C1	44.0	60.7	22.6	-

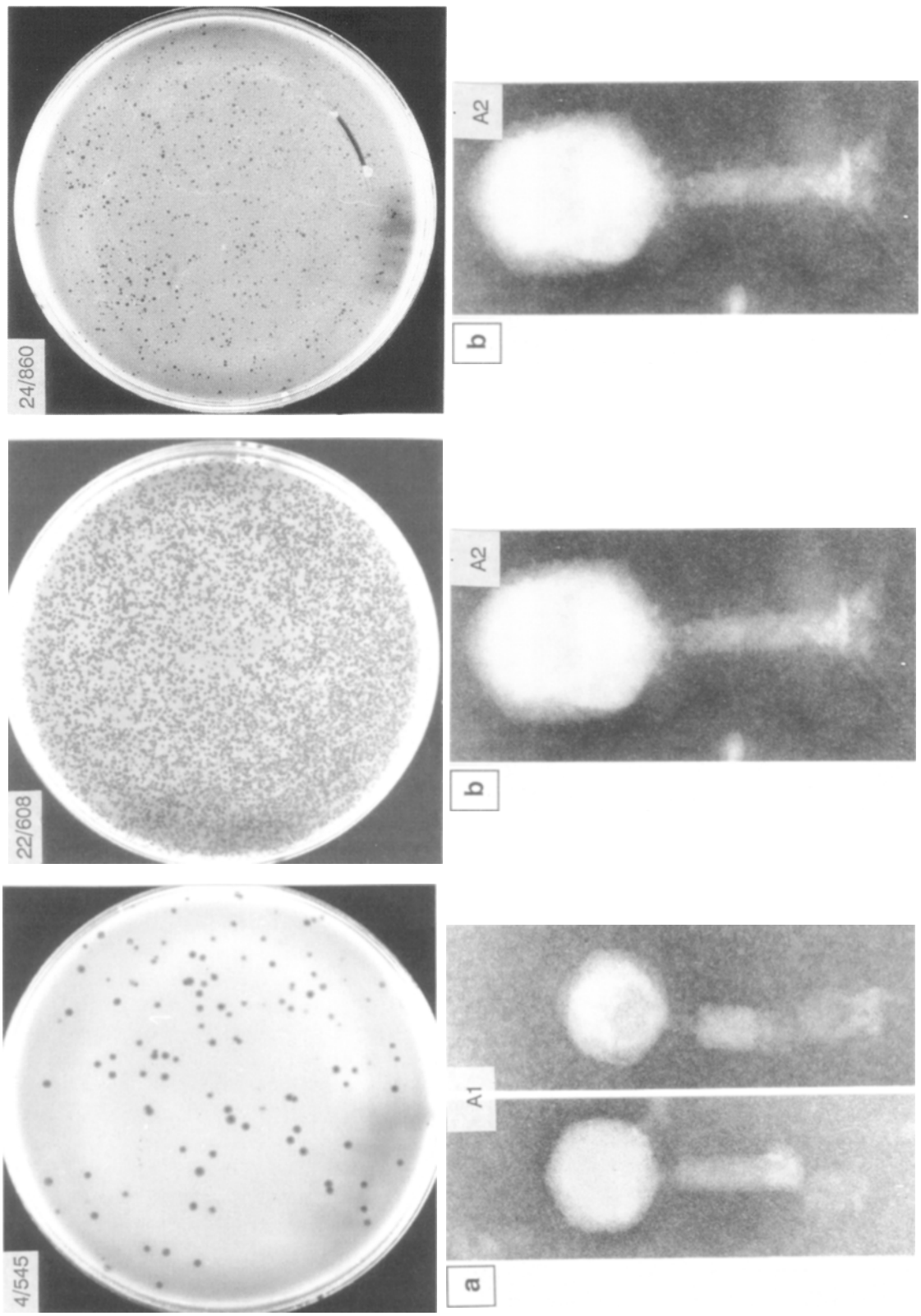
[a]Morphotype according to Ackermann and Eisenstark 1974.
[b]Mean of 15 particles measured.

DISCUSSION

The newly established set of *Proteus* phages included tailed phages which did not differ in morphology from the hundreds of enterobacterial phages described in the literature (Ackermann and DuBow 1987*b*). The phages fell into three families, namely, *Myoviridae*, *Siphoviridae* and *Podoviridae* (Francki *et al.* 1991) and had A1, A2, B1 and C1 morphotypes (from Ackermann and DuBow 1987*a*). The heads of some phages were isometric or elongated (A2 type) and tails were long, contractile or non-contractile, or short. Similar morphotypes were reported by Ackermann and Gerschman in *Salmonella* phages in which, however, the authors occasionally found also morphotypes with elongated heads of A3 and B2 types.

The nucleic acid of phage genomes was determined as double-stranded DNA in 19 out of 22 phages studied (Fig. 2). The remaining 3 phages (22/608, 30/860 and 5/742) showed chromosomes composed of DNA which was sensitive to S1 nuclease while resisting the action of restriction endonucleases. This implies the presence of single-stranded DNA.

The above *Proteus* phages were successfully used in our laboratory for *Proteus* phage typing. However, the results of this study did not allow us to determine the degree of difference, relation or identity of the phages investigated. Further studies involving serotyping, ribotyping and DNA-DNA hybridization will be necessary to provide this information.


24/860
b
A2
22/608
b
A2
4/545
a
A1

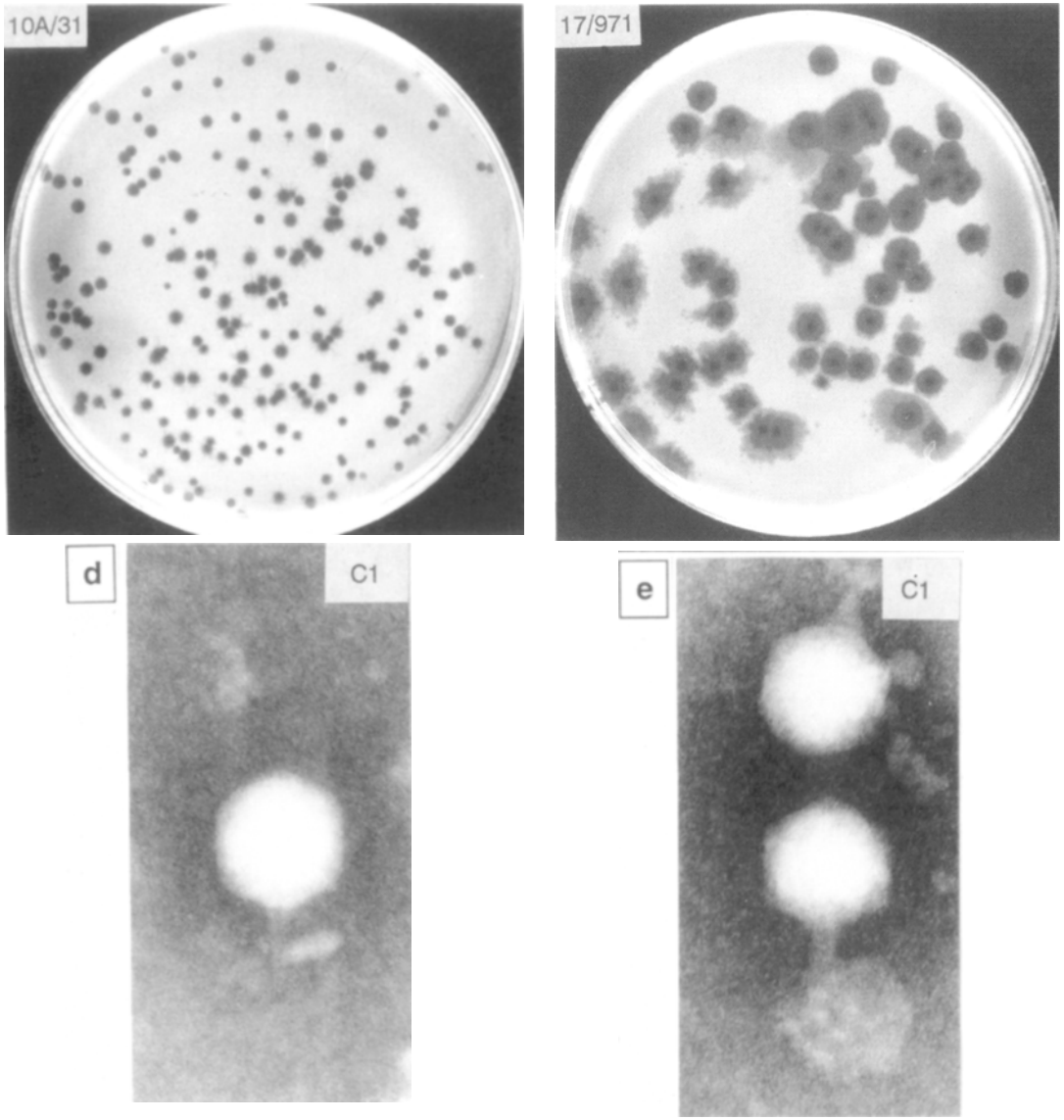


Fig. 1. Bacteriophages **4**/545, **22**/608, **24**/860, **10A**/31, **17**/971.
Top series: plaque morphology.
Bottom series: electron-microscopic morphology (morphotype is given); **a**, *left* – non-contracted tail; **a**, *right* – contracted tail;
original magnification: 326 500×;
actual magnification: 277 525×.

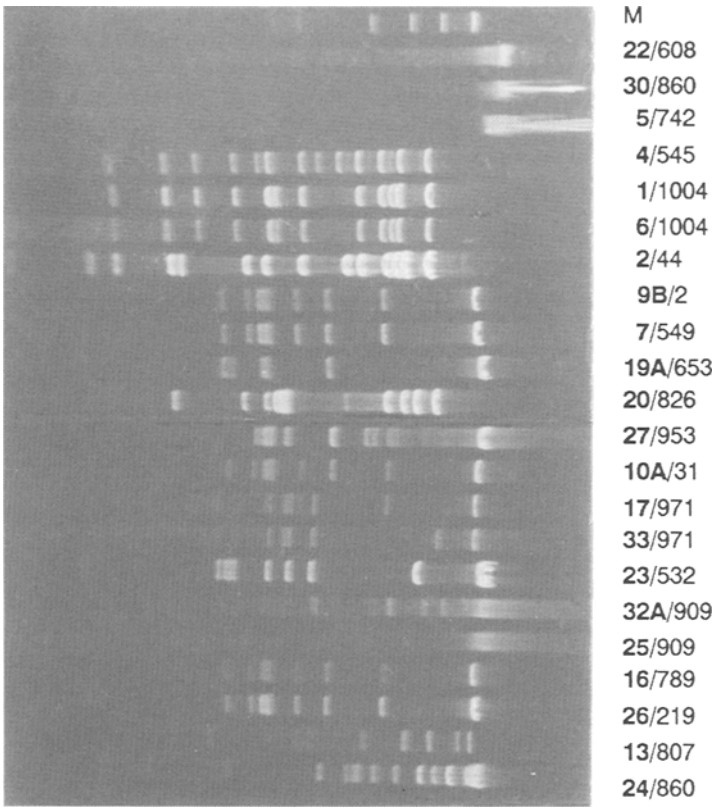


Fig. 2. DNA of 22 *Proteus* phages (1/1004 – 33/971) digested with restriction endonuclease *Hin*dIII; M – molecular size standard (λ DNA digested with *Hin*dIII).

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