The Morphology of *Proteus* Bacteriophages

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(Received 1 March 1965)

SUMMARY

The morphology of 28 bacteriophages active on members of the *Proteus*–*Providencia* group of organisms was examined by a negative staining technique. Examples of the ‘T-even’, T3 and P22 morphologies were encountered as well as phages with non-contractile cross-striated tails resembling a group of coliphages. Others resemble staphylococcal and pseudomonas phages. Two phages (9, 7479) with octahedral capsids were encountered. A temperate *Proteus mirabilis* phage (12/57) and a temperate *P. morganii* phage (10041/2815) differ from phage P 22 in that they possess collars. A *P. rettgeri* phage (7480 b) is unique in that it possesses a head with a regular hexagonal outline, a collar and a non-contractile tail while a *Providencia* phage (9266) is believed to be the largest phage active on Gram-negative bacteria.

INTRODUCTION

The morphology of *Proteus* bacteriophages was first examined by Ruska (1943, 1950). He examined shadow-cast preparations and recorded the dimensions of tadpole-shaped phages. Coetzee (1958) examined shadow-cast preparations of phages active on *Proteus vulgaris* and *P. mirabilis*. They were also tadpole shaped. In a paper on defective lysogeny Taubeneck (1963) published photographs of shadow-cast preparations of a tadpole-shaped phage derived from a lysogenic strain of *P. mirabilis*. Other illustrations in the paper show tail cores surrounded by contractile sheaths adsorbed to *P. mirabilis* and *P. vulgaris* strains. These tails were liberated by defective lysogenic strains and no head structures were visible.

In the family Enterobacteriaceae bacteria of the *Proteus* and *Providencia* groups share the distinction that they alone possess a phenylalanine deaminase (Shaw & Clarke, 1955). They display many intra- and inter-group differences (Ewing, 1958) and their classification is uncertain. Rauss (1962) would allocate them tribal rank in the family with genera *Proteus*, *Morganella*, *Rettgerella* and *Providencia*. Topley & Wilson’s *Principles* (1964) recognize ‘*vulgaris*, *mirabilis*, *morganii* and *rettgeri*’ species in a genus *Proteus* of the tribe Enterobacterieae. They do not commit themselves on the position of the *Providencia* group. Kauffmann (1951) has combined ‘*mirabilis*’ and ‘*vulgaris*’ strains in a species ‘*hauseri*’. Falkow, Ryman & Washington (1962) suggest that *P. morganii* is distinct genetically, possessing a guanine + cytosine molar content of 50 %, compared with other *Proteus* and *Providencia* strains, where content is about 40 %. We decided to study the structure of phages active on the above organisms as an approach to the taxonomy of both the phages and their hosts.

METHODS

Bacteriophages and host organisms. Phages were isolated from sewage by the enrichment technique of Adams (1959) or were derived from lysogenic strains, spontaneously or by ultraviolet induction. All but 4 of the phages have been used in other investigations and are listed in Table 1 together with the method of isolation, indicator host and reference to the literature. Sewage phages 50, 67b and

Table 1. *Sources of bacteriophages*

Phages	Isolation	Indicator	Reference
<i>Proteus hauseri</i>			
9	Sewage	<i>P. vulgaris</i> 9	Coetzee, 1958
15	Sewage	<i>P. vulgaris</i> 15	
65	Sewage	<i>P. vulgaris</i> 65	
67b	Sewage	<i>P. vulgaris</i> 67	See Methods
78	Sewage	<i>P. vulgaris</i> 78	Coetzee, 1958
12	Sewage	<i>P. mirabilis</i> 12	
14	Sewage	<i>P. mirabilis</i> 14	
57	Sewage	<i>P. mirabilis</i> 57	Coetzee & Sacks, 1959, 1960 a, b
34/13	<i>P. mirabilis</i> 34	<i>P. mirabilis</i> 13	
12/57	<i>P. mirabilis</i> 12	<i>P. mirabilis</i> 57	
$\frac{24}{25}$ /14	<i>P. mirabilis</i> 24, 25	<i>P. mirabilis</i> 14	See Methods
13 vir	<i>P. mirabilis</i> 13	<i>P. mirabilis</i> 13	
<i>P. morganii</i>			
50	Sewage	<i>P. morganii</i> M50	See Methods
5845	Sewage	NCTC 5845	Coetzee, 1963 a
10041/2815	NCTC 10041	NCTC 2815	
47	M47	<i>P. morganii</i> M235	
Providence			
9248	Sewage	NCTC 9248	Coetzee, 1963 b
9266	Sewage	NCTC 9266	
9211/9295	NCTC 9211	NCTC 9295	
9213/9211 a, b	NCTC 9213	NCTC 9211	
9000/9402	NCTC 9000	NCTC 9402	
<i>P. rettgeri</i>			
8893	Sewage	NCTC 8893	Coetzee, 1963 c
7480	Sewage	NCTC 7480	
7480 b	Sewage	NCTC 7480	See Methods
7479	Sewage	NCTC 7479	Coetzee, 1963 c
7478/325	NCTC 7478	R325	
7476/322	NCTC 7476	R322	

7480b productively lyse *Proteus morganii* strain M50, *P. vulgaris* strain 67 and *P. rettgeri* NCTC7480 respectively. Phage 13 vir is present in plaques which appear spontaneously on cultures of *P. mirabilis* strain 13. Organisms of this strain cannot be freed of the phage by suspension in high-titre phage antiserum, and this phage is therefore regarded as a virulent mutant of a temperate phage of strain 13. The temperate phage has not yet been demonstrated. The general phage techniques were those of Adams (1959).

Media. The nutrient agar was that of Adams (1959) and the broth was that of Coetzee & Sacks (1960c).

Electron microscopy. High-titre phage lysates of sewage phage were prepared by infecting young aerated broth cultures from single plaques. Temperate phages were

prepared by the modification (Adams, 1959) of the double-agar-layer method of Hershey, Kalmanson & Bronfenbrenner (1943). The lysates were purified and concentrated by differential centrifugation (de Klerk, Coetzee & Fourie, 1965). The purified phages (plaque-forming titres about 1×10^{11} /ml.) were suspended in 0.1 M-ammonium acetate (pH 7.2). The negative staining method of Brenner & Horne (1959) was used. Phage suspensions in neutral potassium phosphotungstate were mounted on carbon support films by the spreading technique (Bradley, 1962) and examined with a Philips EM200 electron microscope. In the absence of spontaneous contraction of tail sheaths, hydrogen peroxide (3%, v/v) and ethyl alcohol (10%, v/v) were added to phage suspensions to cause possible sheath contraction (Kellenberger & Arber, 1955).

RESULTS

Dimensions of the bacteriophages examined are listed in Table 2.

Table 2. *Dimensions (in Å) of Proteus bacteriophages*

Phage	Head*	Tail		Core width	Overall length
		Length	Width		
<i>Proteus hauseri</i>					
15	518	148	222	—	666
34/13	630	167	204	—	797
65	518	148	222	—	666
12/57	589	185	204	—	774
9	826	1837	89	—	2663
13 <i>vir</i>	600	1019	167	60	1619
57	619	882	178	56	1501
67 b	589	981	174	74	1570
78	611	889	163	60	1500
12	593	2163	115	—	2756
14	660	2045	115	—	2705
$\frac{3.4}{2.5}/14$	715 × 604	1715	108	—	2430
<i>P. morganii</i>					
50	970 × 670	1252	159	52	2222
5845	1022 × 763	1252	156	74	2274
10041/2815	544	182	167	—	726
47	556	1481	85	—	2037
Providence					
9248	611	185	—	—	796
9266	1193 × 822	2152	248	96	3345
9213/9211 a	626	1696	137	—	2322
9213/9211 b	608	152	204	—	760
9211/9295	533	148	196	—	681
9000/9402	682	2304	148	—	2986
<i>P. rettgeri</i>					
8893	926 × 741	1296	178	74	2222
7480	634	2185	126	—	2819
7480 b	367	330	63	—	697
7479	737	1852	85	—	2589
7478/325	604	1741	115	—	2345
7476/322	533	1600	77	—	2133

* Dimensions between apices are given, or apex to tail-joint and width. Figures are the mean of 8 to 12 measurements.

Phages active on Proteus hauseri strains

Phages 15, 34/13, 65. (Pl. 1, figs. 1, 2.) These are similar in morphology. Phages 65 and 34/13 are presented. The heads have hexagonal outlines and attached to one of the angles by means of a short neck is a base-plate with a number of pins. These phages resemble salmonella phage P 22 (Anderson, 1960) and pseudomonas phage 12B (Bradley, 1963*a*) but phages 15 and 65 are slightly smaller.

Phage 12/57. (Pl. 1, fig. 3.) This phage resembles those just mentioned but possesses a delicate collar about 200 Å wide, around the short neck. No tail fibres were seen.

Phage 9. (Pl. 1, fig. 4.) The head of this phage is large, hexagonal in outline and octahedral in shape. The tail is thin, cross-striated, non-contractile and ends in a point with tail fibres. It resembles pseudomonas phage 24 (Bradley, 1963*a*), phage 1C (Bradley, 1962) and phages F1 (Bradley, 1963*b*) and WAK/2 (Bradley, 1964).

Phages 13 vir, 57, 67b, 78. (Pl. 1, figs. 5, 6, 7.) These phages have a similar morphology and 13 vir, 57 and 78 are shown. The head is regular hexagonal in plan view. The tail consists of a core surrounded by a contractile sheath to which is attached an inconspicuous base-plate, probably with 6 prongs. Cross-striations are visible on some of the sheaths with a periodicity of about 40 Å. These phages resemble Vi phage 1 (Bradley & Kay, 1960) although their heads are smaller.

Phages 12, 14. (Pl. 1, fig. 8.) These phages are similar and correspond to the group of 'smaller phages without contractile tails' of Bradley (1963*b*). Phage 12 is shown. Their heads are polygonal and often hexagonal in outline. The tails are non-contractile and markedly cross-striated with a periodicity of about 40 Å. A small bundle of fibres is present at the tail tip.

Phage 24/14. (Pl. 1, fig. 9.) This phage has a polyhedral head slightly longer than wide which closely resembles that of phage ZG/3A (Bradley, 1964). It has a non-contractile tail with cross-striations about 30 Å apart. No tail fibres have been observed.

Phages active on strains of Proteus morganii

Phages 50, 5845. (Pl. 1, fig. 10; Pl. 2, figs. 11, 12.) These phages are similar in shape. The dimensions and appearance of the heads suggest that they may be bipyramidal hexagonal prisms and the tails consist of cores surrounded by cross-striated contractile sheaths. A base-plate with pins moves with the sheath. Tail fibres are present and in some photographs they form a fibrous network which extends to the collar (Bradley, 1963*b*). The collars have a width of 360 Å and a periodicity of the sheaths of this group is about 40 Å. In Pl. 2, fig. 11, is a separate rosette-shaped structure which may be either a base-plate or a collar. These phages resemble phage T4, which is reported to possess a bipyramidal hexagonal head (Bradley, 1963*b*).

Phage 10041/2815. (Pl. 2, fig. 13.) This phage is similar to but slightly smaller than *P. hauseri* phage 12/57. The collar above the base-plate can be clearly seen on one of the phages in the figure. No tail fibres have been observed.

Phage 47. (Pl. 2, figs. 15, 18.) This phage has a head with a hexagonal outline. The tail is non-contractile and cross-striated. It has no neck or collar, tail fibres have not been identified and it resembles the SBL group of typhoid phages (Bradley, 1963*b*).

Phages active on Providence strains

Phage 9248. (Pl. 2, fig. 14.) The head of the phage is polyhedral and hexagonal in plan view. It has a wedge-shaped tail attached to one corner and resembles coliphage T3. It also resembles brucella phages (Brinley-Morgan, Kay & Bradley, 1960; McDuff, Jones & Wilson, 1962) and a group of pseudomonas phages (Bradley, 1963*a*).

Phage 9266. (Pl. 2, figs. 16, 17.) This large phage has a head which is hexagonal in outline and resembles those of the T-even phages. The tail core is surrounded by a contractile sheath with cross-striations. No base-plate is evident. Attached to the tail sheath and visible in the contracted state (Pl. 2, fig. 16) are 6 short spikes and a tangled mass of fibres. It has a neck but no collar.

Phage 9213/9211a. (Pl. 2, fig. 19.) The phage has a head with a regular hexagonal outline in certain views. The tail is non-contractile, has cross-striations and ends in a rosette-shaped structure. It resembles staphylococcal phages 187, 52 (Bradley, 1963*a*) and pseudomonas phages Pc, Pz (Bradley & Kay, 1960).

Phages 9213/9211b, 9211/9295. (Pl. 3, figs. 20, 21). These phages resemble the *P. hauseri* groups 34/13, 15, 65 and 12/57 and the *P.morganii* phage 10041/2815. On occasions a rod 30–40 Å wide which projects about 150 Å beyond the base-plate was seen, but could not be consistently demonstrated. No tail fibres were seen.

Phage 9000/9402. (Pl. 3, fig. 23.) The outline of the head is a regular hexagon. It has a long non-contractile tail with cross-striations and some fibres at the tip. This phage is slightly longer than the *P. hauseri* phages 12, 14, but smaller than the C₁, F₁ group of coliphages (Bradley, 1963*b*).

Phages active on strains of Proteus rettgeri

Phage 8893. (Pl. 3, fig. 26.) The structure is similar to *P.morganii* phages 50, 5845.

Phage 7480. (Pl. 3, fig. 22.) This phage is similar to Providence phage 9000/9402.

Phage 7480b. (Pl. 3, fig. 25.) The head has a regular hexagonal outline. A short tail is attached to one corner by a neck which is surrounded by a collar. The tail could not be made to contract by the methods used and cross-striations were not seen. Despite repeated efforts the detailed structure of the tail-tip could not be resolved. The collar is 100 Å wide.

Phage 7479. (Pl. 3, fig. 24.) This phage resembles *P. hauseri* phage 9 although the octahedral head is slightly smaller.

Phage 7478/325. (Pl. 3, fig. 27.) This phage resembles *P. hauseri* phage 12, 14, *P.morganii* phage 47, Providence phage 9000/9402 and *P. rettgeri* phage 7480. The dimensions are similar to those of T5 (Bradley & Kay, 1960).

Phage 7476/322. (Pl. 3, fig. 28.) The phage head is hexagonal in outline. The tail is thin, cross-striated and measures about 30 Å less in width than tails of the SBL group (Bradley & Kay, 1960).

Many of the capsids have an uneven rough appearance suggestive of arrays of capsomeres (Bradley, 1964) but neither their shape nor their packing arrangements could be determined. Apart from those whose heads are obviously octahedral, the head shapes of the phages could not be determined. We have indicated those which may have bipyramidal shapes and the remainder are possibly icosahedra.

DISCUSSION

Undescribed varieties of phage have been found. The *Proteus mirabilis* transducing phage 12/57 differs from its salmonella counterpart P22 in that it has a delicate collar. The same applies to *P. morganii* phage 10041/2815. Phage 7480b is unique in that it has a collar, a non-contractile tail and a head with a regular hexagonal outline. Phage ZG/3A described by Bradley (1964) is similar, but has a bipyramidal prismatic head. The overall dimensions of phage 9266 are larger than those of phage P1 (Anderson, 1960) (which is described as the largest phage yet examined (Hayes, 1965)) though some staphylococcal phages have longer tails (Bradley & Kay, 1960). Phage 13 *vir* has a unique morphology for a phage derived from a lysogenic bacterium. The nearest to it are phages P1, P2 (Anderson, 1960), which are much larger, lack tail pins and have less conspicuous tail fibres. The original lysate of phage 9218/9211 (Coetzee, 1963*b*) was thought to be pure, but it has yielded two morphological types. There is a possibility that one of the phages may be derived from the indicator strain NCTC 9211 although this strain has not been proved lysogenic.

It is not known how the P22 group of phages infect their hosts (Anderson, 1960). The finding here of phages which resemble P22 but have collars may eventually cast light on the process. Tail fibres were never observed in these phages. The nature of the structure which projects beyond the base plates of phages 9218/9211*b* and 9211/9295 is not known. It could not always be demonstrated and may be nucleic acid.

Providence phage 9248 resembles coliphage T3. Its head shape could not be established but it may be octahedral like that of phage T3 (Bradley, 1963*b*). Two other phages (9, 7479) of this series possess octahedral capsids. Caspar & Klug (1962) have cast doubt on the octahedral shapes of phage capsids but Bradley (1963*b*; 1964) has clearly demonstrated such capsids among coliphages.

Good correlation between the morphological and serological types of phages have been recorded (Bradley, 1963*a*; de Klerk, Coetzee & Theron, 1963; de Klerk *et al.* 1965). Phages 15, 65, which are morphologically identical, are related serologically but the identical structural types 57, 78 differ serologically and in other aspects (Coetzee, 1958). The bacteriophages studied show considerable morphological variation and identical types grow on representatives of most of the groups of organisms. This lack of morphological specificity is a great problem in bacteriophage taxonomy (Dawson, Smillie & Norris, 1962; Bradley, 1963*b*). Results also diminish the hope of finding a particular morphological type with a predilection for one or other of these groups of organisms like that of the RNA and filamentous phages for male strains of *Escherichia coli* (Bradley, 1964).

One of us (J.N.C.) is in receipt of a grant from the South African Council for Scientific and Industrial Research.

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EXPLANATION OF PLATES

The magnification in all Figs. is $\times 270,000$. All phages in ammonium acetate and phosphotungstate. Figs. 1-9. Phages active on *Proteus hauseri* strains. Figs. 10-13, 15, 18: phages active on *P. morganii* strains. Figs. 14, 16, 17, 19-21, 23: phages active on *Providencia* strains. Figs. 22, 24-28: phages active on *P. rettgeri* strains.

PLATE 1

- Fig. 1. Phage 65.
- Fig. 2. Phage 34/13.
- Fig. 3. Phage 12/57.
- Fig. 4. Phage 9.
- Fig. 5. Phage 13 *vir*.
- Fig. 6. Phage 78.
- Fig. 7. Phage 57.
- Fig. 8. Phage 12.
- Fig. 9. Phage $\frac{2\frac{1}{2}}{15}$ /14.
- Fig. 10. Phage 50.

PLATE 2

- Figs. 11, 12. Phage 5845.
- Fig. 13. Phage 10041/2815.
- Fig. 14. Phage 9248.
- Fig. 15. Phage 47.
- Figs. 16, 17. Phage 9266.
- Fig. 18. Phage 47.
- Fig. 19. Phage 9213/9211 a.

PLATE 3

- Fig. 20. Phage 9213/9211 b.
- Fig. 21. Phage 9211/9295.
- Fig. 22. Phage 7480.
- Fig. 23. Phage 9000/9402.
- Fig. 24. Phage 7479.
- Fig. 25. Phage 7480 b.
- Fig. 26. Phage 8893.
- Fig. 27. Phage 7478/325.
- Fig. 28. Phage 7476/322.

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