

## Taxonomic changes in tailed phages of enterobacteria

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**Summary.** Out of 136 new phages, 80 (59%) are classified into 23 species according to morphology and physicochemical properties. Six new species are described and species  $\beta 4$ , from a previous classification scheme, is renamed T1. The morphology of 36 phage species is schematically represented.

### Introduction

Tailed phages of enterobacteria belong to three virus families: *Myoviridae* or phages with contractile tails, *Siphoviridae* or phages with long, noncontractile tails, and *Podoviridae* with short tails [4, 30]. Virion heads vary in shape and are isometric, moderately elongated, or long and narrow. Each family may thus be divided into three morphotypes according to head length [4].

Bacteriophages include about 4 500 isolates of known morphology and constitute the largest viral group known. As many as 750 of these viruses are tailed phages of enterobacteria [2]. Their classification is thus paramount to

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phage taxonomy. A first survey of these phages was published in 1976 [1]. About 250 phages were classified into 24 species by morphology, serology, and physicochemical properties. A second survey, published in 1987, included 30 species [5]. Since then, about 140 new phages were described or deposited in the collection of one of us (H.-W. A). Some of them were clearly new entities, warranting an update of the present enterobacteriophage classification.

### Materials and methods

A total of 136 phages were surveyed, including viruses of *Enterobacter* (9), *Escherichia* (40), *Klebsiella* (2), *Proteus* (22), *Salmonella* (44), *Serratia* (2), *Xenorhabdus* (2), and *Yersinia* (15). All but 6 were from the literature. The unpublished *Salmonella senftenberg* phages SasL1 to SasL6 were isolated by H. Singh (Postgraduate Institute for Medical Education and Research, Chandigarh, India) and were deposited in the Félix d'Hérelle Center for safekeeping. Phages SasL1 to SasL6, *Enterobacter* phages WS-EP20 and WS-EP57 [27], *Serratia* phage 3M [36], and *Yersinia* phage YerA41 [41] were examined or controlled in the electron microscope. Phages were propagated as agar surface cultures [4] on trypticase-soy agar (Difco, Detroit, MI, U.S.A.) at 37°C. Lysates were sterilized by filtration through membrane filters of 45 µm pore size (Millipore Co., Bedford, MA, U.S.A.). Phages were prepared for electron microscopy as previously described [3]. Dimensions were measured on 20 phosphotungstate-stained particles per phage. Diameters of isometric heads were measured between opposite apices.

### Results and discussion

#### *Phage classification*

The 136 surveyed phages belonged to the *Myoviridae* (42 isolates), *Siphoviridae* (55 isolates), and *Podoviridae* (39 isolates). Eighty phages (59%) were classified into 23 species, six of which were new (Table 1). The success rate of classification was highest in myoviruses because of their relatively characteristic morphology. Similarly, siphoviruses and podoviruses with elongated heads were readily classified. The classification of new isolates into established phage species was generally based on morphological criteria. Tentative identifications are indicated in parentheses. A number of phages could not be identified at all for lack of serological or DNA-DNA hybridization data, or because of poor electron micrographs. Coliphage  $\phi$ R73, a myovirus with a retroelement and a relative of coliphage P4, was not classified because it was defective [22, 41]. Two other myoviruses [23] were not classified because they resembled "killer-particles" of *Bacillus* [4] and were clearly contaminants. Further nonclassified phages may be found in references 8, 9, 11, 16, 20, 23, 25, 31–34, 39, 40, 42, and 43.

#### *New phage species*

Four species were found to belong to the *Myoviridae* and two species to the *Siphoviridae* phage families, respectively. Capsids were icosahedral and smooth and did not show capsomers, while tails showed transverse striations. Species were named after their best-studied (or only) representatives, which were also

**Table 1.** Classification of new phages

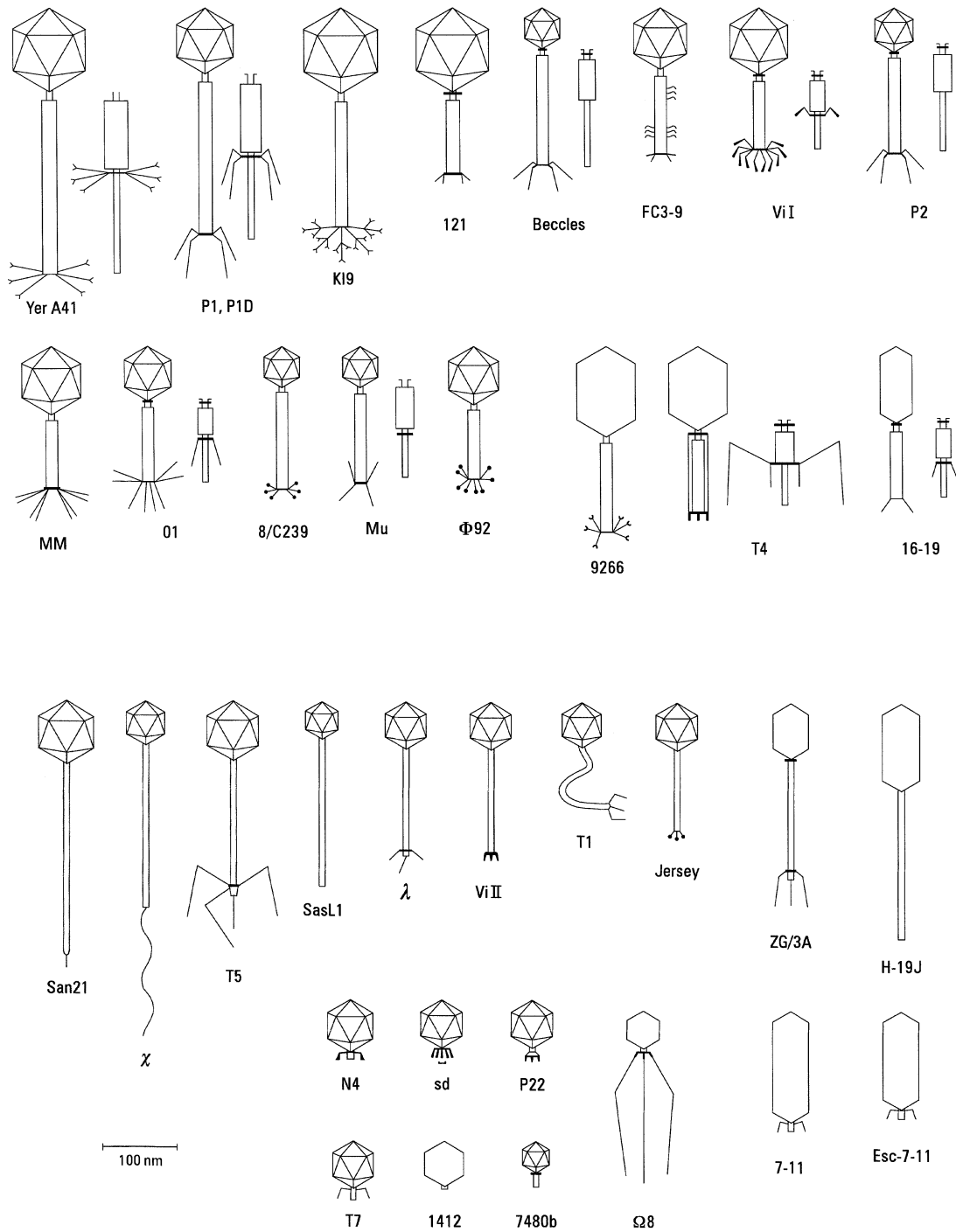
Family	Species	Phages	Host genus	References
<i>Myoviridae</i>	FC3-9	WS-EP57	<i>Enterobacter</i>	27
	MM*	MM	<i>Escherichia</i>	29, 46
	Mu	B278	<i>Escherichia</i>	18
	O1	(HI8A, HI8B, S1)	<i>Escherichia</i>	24, 44, 45
	P1D*	P1D	<i>Escherichia</i>	7
	P2	PsP3	<i>Escherichia</i>	10, 12
	ViI	15, 17, (CT); 3M	<i>Salmonella, Serratia</i>	6, 36, 42
	YerA41*	YerA41, (YerA7)	<i>Yersinia</i>	40
	8/C239*	8/C239	<i>Yersinia</i>	24
	T4	WS-EP32, -94, -96; Ab48, FC3-10; 20, 22; 2/F2852	<i>Enterobacter, Escherichia, Klebsiella, Proteus, Yersinia</i>	14, 15, 25, 27, 39
<i>Siphoviridae</i>	16-19	11, 12	<i>Salmonella</i>	6
	Jersey	3, 7, 8	<i>Salmonella</i>	6
	San21*	21	<i>Salmonella</i>	6
	SasL1*	SasL2 to SasL5	<i>Salmonella</i>	This work
	T1	S2; Hi, 102, 174, 150, 168	<i>Escherichia</i>	21, 44, 45
	T5	14 phages	<i>Salmonella</i>	6
	λ	HK253	<i>Escherichia</i>	35
	χ	WS-EP20, -26, -28; 1	<i>Enterobacter, Salmonella</i>	6, 27
	ZG/3A	10 phages; 27	<i>Escherichia, Salmonella</i>	6, 15
	H-19J	SasL6	<i>Salmonella</i>	This work
<i>Podoviridae</i>	N4	(WS-EP13, -19); CF0103; 10	<i>Enterobacter, Escherichia, Salmonella</i>	6, 27, 38
	T7	WPK; SP6, 5; 4, 7	<i>Escherichia, Salmonella, Yersinia</i>	6, 13, 26
	7-11	SNT-3; 1/M61	<i>Salmonella, Yersinia</i>	25, 42

Phage species are listed in the order of Fig. 1. New species are indicated by asterisks. Tentatively identified phages are in parentheses

considered as type viruses. The morphology of the six new species is illustrated by scale drawings (Fig. 1); in addition, species SasL1 and YerA41 are illustrated by electron micrographs. Particle dimensions and other properties of phage species and the origin of type viruses are indicated in Table 2.

#### *Myoviridae* (tail contractile)

1. Species YerA41 (Fig. 2) is the largest enterobacterial phage known. Particles have very large heads of about 110 nm in diameter. Tails consist of a neck of  $10 \times 8$  nm, a sheath which measures  $250 \times 20$  nm in the extended and  $90 \times 30$  nm in the contracted state, and feathery, approximately 70 nm long tail fibers that seem to be attached to the lower end of the tail sheath.
2. Species P1D is morphologically indistinguishable from phage P1, except for its longer tail which measures 239 instead of 226 nm in length [7].
3. Species MM has a large head and a relatively short tail with 12 straight tail fibers that tend to aggregate pairwise. Phage MM is well characterized. It

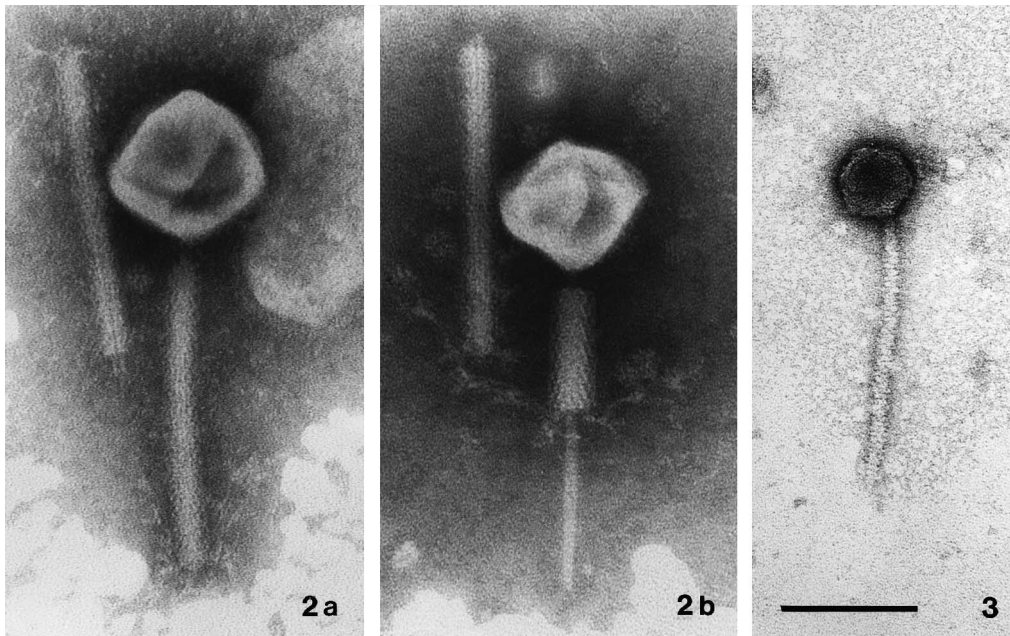


**Fig. 1.** Species of tailed enterobacterial phages. Species are grouped by family, head shape, and particle size. Modified from [5], pp 96–97 (with permission)

Table 2. Properties of new species and origin of type viruses

Family	Species	Dimensions, nm		Particulars	Common host	Isolation			References
		Head	Tail			Site	Year	From	
<i>Myoviridae</i>	YerA4	110	250 × 20	Large size, tail fibers feathery	<i>Y. ruckeri</i>	Guelph, Ont.,	1984?	Sewage	40
	P1D	87	226 × 18	P1-like, temperate	<i>E. coli</i>	Quebec, Qc,	1991	Lysogenic strain	7
	MM	92	103 × 15	Six pairs of short straight tail fibers	<i>E. coli</i>	Therwil, Basel, Switzerland <sup>a</sup>	1983 <sup>a</sup>	Sewage	29, 46
	8/C239	58	140 × 15	Short, club-shaped tail fibers	<i>Y. enterocolitica</i>	Ste-Anne-de-Bellevue, Qc, Canada	1976	Human feces	25
<i>Siphoviridae</i>	San21	84	260 × 8	Large size, aberrant heads	<i>S. anatum</i>	Orono, ME, U.S.A.	1970?	Sewage	6
	SasL1	56	190 × 7	Small head, rigid tail, temperate	<i>S. senftenberg</i>	Kasauli, India	1992	Lysogenic strain	This work

<sup>a</sup>Dr. M. Wurtz, Basel, pers. comm.



**Fig. 2.** Phage YerA41 with extended tail (a) or contracted (b). The rods are isolated tails, Phosphotungstate,  $\times 297\,000$ . **Fig. 3.** Phage SasL1, uranyl acetate,  $\times 297\,000$ ; bar: 100 nm

contains double-stranded DNA with a length of 131 kbp and a G-C content of 50%. Intact particles have a buoyant density of 1.515 g/ml in CsCl, a mass of 144 Mda, and a DNA content of 62%. Capsid and tail contain two major structural proteins each [30].

4. Species 8/C239 resembles coliphages Mu and P2, but differs from them by short, club-shaped spikes at the tail end [25].

#### *Siphoviridae* (tails long and noncontractile)

1. Species San21, originally designated as "21", was renamed after its host, *Salmonella anatum*. The species represents the largest known enterobacterial phage with a long, noncontractile tail. In addition to normal particles, lysates contain numerous aberrant, mottled heads of regular or irregular shape [6]. The reason for the generation of these aberrant particles is unknown.
2. Species SasL1 (Fig. 3) has no morphological particulars. Heads are relatively small and tails are rigid. The species is proposed only because phages of this type differ morphologically from already established enterobacterial phage species.

P1D and the very similar *Aeromonas* phage 43 [7] may be considered as tail length variants and mere subspecies of P1. We prefer to classify them as separate species because the P1 group has been given genus rank at the Xth International Congress of Virology (August 1996, unpubl.) and because this

classification gives more flexibility in the classification of tailed phages. Indeed, these viruses are extremely heterogeneous with respect to DNA homology and serological properties, so that several morphologically homogeneous enterobacterial phage species appear as morphospecies rather than as homogeneous genetic groups. For example,

1. The  $\chi$  species of enterobacterial phages consists of three DNA homology groups (F. Grimont, Pasteur Institute, Paris, pers. comm.).
2. The rare C3 morphotype has only 13 representatives among about 4 400 tailed phages [2]. However, a comparison of six enterobacterial phages of this type has shown two head length variants and no less than three DNA homology and seroneutralization groups [17].
3. The T4 group includes at least six DNA homology and seroneutralization groups [28]. The genome polymorphism of T-even type phages appears even greater if individual chromosomal regions are compared, for example those coding for internal proteins [37] or tail fibers [19].

#### *Renaming a phage species*

The previous classification schemes of enterobacterial phages included a species  $\beta_4$ , named after its first known representative, and characterized by an extremely flexible, frequently curled-up tail with short terminal fibers. The group included 12 phages of the *E. coli-Klebsiella-Shigella*-group [2, 4]. At the same time, the well-known coliphage T1 remained unclassified because of its uncharacteristic morphology. The examination of phosphotungstate-stained phage T1 and a series of related viruses showed that all of them had the same unusually flexible tail [22]. We confirmed this observation and found that the tail of phage T1 appears rigid after uranyl acetate-staining only. We conclude that phosphotungstate produces, in this case, a diagnostically valuable staining artifact, that phages  $\beta_4$  and T1 are related, and that the “ $\beta_4$  group” should be renamed “T1 species” after its best-known member.

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