The Fine Structure of *Lactobacillus* Bacteriophages

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SUMMARY

Twelve phages isolated from sewage and active on lactobacilli were examined by electron microscopy with a negative-staining technique. Those phages active on *Lactobacillus fermenti* (heterofermentative) possess icosahedral heads and sheathed tails which end in base-plates and pins. Those phages active on *Lactobacillus casei* (homofermentative) differ in that their heads are octahedral or icosahedral and they possess collars. The overall length of all the phages is similar and their base-plates remain attached to the sheaths when these contract. No tail fibres were seen. A temperate *Lactobacillus fermenti* phage was also examined. It has a small hexagonal head and a long unsheathed tail which ends in a star-shaped structure.

INTRODUCTION

An electron micrograph of a shadow-cast preparation of a phage active on *Lactobacillus casei* was presented in an M.S. thesis (Walter, 1958). De Klerk, Coetzee & Theron (1963) examined shadow-cast preparations of a number of phages active on lactobacilli. These phages are morphologically heterogeneous and it was decided to study their fine structure.

METHODS

Media. Media used were MRS broth and agar (de Man, Rogosa & Sharpe, 1960).

Phages. These phages were isolated from sewage and were as follows: nos. 41, 69, 206, 222, 222*a*, 315, 514 and 517 active on *Lactobacillus fermenti* strains; 300, 316, 356 and 780 active on strains of *L. casei* (de Klerk *et al.* 1963). The temperate phage 535/222*a* (Coetzee & de Klerk, 1962) was also examined.

Electron microscopy. Lysates of the sewage phages were prepared, purified and concentrated as previously described (de Klerk *et al.* 1963). High-titre lysates of the temperate phage were prepared by a double agar layer method (Hershey, Kalmanson & Bronfenbrenner, 1943). The purified phages (plaque-forming titres about 1×10^{12} /ml.) were suspended in 0.1 M-ammonium acetate (pH 7.2). The negative staining method of Brenner & Horne (1959) was used. Perforated carbon films were prepared on Veco 400 mesh/in. support grids (Sjöstrand, 1956); these were freed from formvar and oil by immersion in redistilled chloroform. The specimens were mounted by the spreading technique (Bradley, 1962) and examined with a Philips EM 200 electron microscope.

RESULTS

Phages active on Lactobacillus fermenti. Plate 1, figs. 1–5, shows *Lactobacillus fermenti* phages 206, 222*a*, 315 and 514. Their heads are composed of capsomeres. Some of the latter show a central core filled with phosphotungstate and are presumably hollow. The arrangement of the capsomeres has not been determined but the shapes of the heads are consistent with that of a regular icosahedron. The tails are composed of thin central cores surrounded by contractile sheaths composed of subunits in a helical arrangement. No collars are present and the terminal tail structures are rosette-like when the sheaths are extended. When the sheaths contract the central cores are exposed. The tail structures then show base-plates carrying no more than six tail pins, attached to the sheaths. No tail fibres were seen. The dimensions of these *L. fermenti* phages are presented in Table 1. The other sewage phages active on *L. fermenti* organisms which were examined had identical features and similar dimensions.

Table 1. *Dimensions (Ångström units) of Lactobacillus bacteriophages*

Phage	Head*	Tail length	Sheath width		Core width	Overall length
			Un-contracted	Contracted		
206	720†	1380	—	180	55	2100
222 <i>a</i>	690	1380	160	200	55	2070
315	720	1480	160	—	—	2200
514	720	1380	—	180	60	2100
300	820	1230	150	190	70	2050
356	820	1270	160	—	—	2120
535/222 <i>a</i>	500	1820	—	—	80	2320

* Dimension from apex to tail joint.

† Figures are the mean of 25 to 30 measurements.

Temperate phage. The temperate phage 535/222*a* possesses a different morphology (Pl. 1, figs. 6, 7). Its head is hexagonal and capsomeres were not seen. The tail is long, hollow and unsheathed and terminates in a star-shaped blob. The dimensions are presented in Table 1.

Phages active on Lactobacillus casei. Plate 2, figs. 8–10, shows *Lactobacillus casei* phages 300 and 356. The heads are composed of hollow capsomeres. The shape of the heads appears to be octahedral but few structural details have been discerned, and the head shape of phage 356 may be icosahedral. These phages also differ from those active on heterofermentative lactobacilli in that collars are present which have the same diameters as the tail sheaths. The remaining tail structures are similar to those of the phages active on heterofermentative lactobacilli. The dimensions of these phages are given in Table 1. Phages 316 and 780 revealed an identical morphology and similar dimensions. The periodicity of the tail sheaths of both groups of sewage phages in the contracted state was 36 Å and 28 Å when extended.

DISCUSSION

A clear distinction is possible between sewage phages active on heterofermentative and homofermentative lactobacilli. Not only do they differ serologically (de Klerk *et al.* 1963) but the latter have collars and probably octahedral heads while the former possess icosahedral heads and lack collars. The collars are thicker than those of coliphage T4 (Bradley, 1963) and resemble the collar of *Bacillus subtilis* phage SP3 (Eiserling & Romig, 1962). The overall length of the two groups of phages is similar, although the heads of those active on homofermentative organisms are larger. Their dimensions are similar to those of many other phages (Shirling, 1956; Bradley & Kay, 1960; Davison, 1963). The sheaths of both groups of phages, when contracted, remain attached to the base-plates. However, methods to produce contraction which may dissociate the base-plate from the contracted sheath (Kellenberger & Arber, 1955) were not used. The tail endings of these phages in the uncontracted state resemble that of *B. subtilis* phage SP8 (Davison, 1963), which is described as a mass of fibres and pins. However, no tail fibres or fibrous network around the sheaths of these phages were detected. This is not exceptional, for tail fibres and the fibrous networks detected in coliphages T2 and T4 have not been described in phages active on Gram-positive organisms (Bradley & Kay, 1960; Eiserling & Romig, 1962; Davison, 1963). The temperate lactobacillus phage differs from the sewage phages in having a smaller head and a longer and unsheathed tail which is distinctly broader than the cores of the other phages. No tail pins or fibres have been identified in the rosette-shaped tail ending of this phage, which is similar to the temperate *B. cereus* phages described by Dawson & Smillie (1962).

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EXPLANATION OF PLATES

The magnification in all figures is $\times 276,000$. All phages in ammonium acetate and phosphotungstate.

PLATE 1

- Fig. 1. Phage 206.
Figs. 2, 3. Phage 222*a*.
Fig. 4. Phage 514.
Fig. 5. Phage 315.
Figs. 6, 7. Phage 535/222*a*.

PLATE 2

- Figs. 8, 9. Phage 300.
Fig. 10. Phage 356.

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