A NEW BACTERIOPHAGE ACTIVE AGAINST A LACTIC STREPTOCOCCUS

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Parmelee, Carr, and Nelson (1949) described bacteriophages active against nine strains of lactic streptococci. They found them so nearly alike in shape and size that they could not differentiate between strains on that basis. Recently we have examined bacteriophages active against eleven strains of lactic streptococci. Of these, all except one are similar to those reported above. We describe below the remaining bacteriophage, which has a distinctly different morphology.

METHODS AND MATERIALS

Bacteriophages active against 10 strains of *Streptococcus cremoris* and 1 strain of *Streptococcus lactis* were supplied by the Dairy Research Institute, Palmerston North, New Zealand, in the form of bacterium-free whey filtrates. They had been freshly prepared under careful control to give maximum titers and were stored in the refrigerator at about 7 C when not in use. They had been selected from a large collection of bacteriophage races and on the basis of their ranges of action on a variety of streptococci (Hunter, 1946) were considered to be almost certainly unrelated.

Specimens were made from the bacteriophage preparations by the following method: (a) Dilute the concentrate 1:8 with physiological saline. (b) Transfer one drop with a platinum wire loop to a collodion-covered specimen grid. (c) Allow drop to dry. (d) Wash grid with successive drops of distilled water. (e) Dry. (f) Shadow with uranium (initial work with gold).

Step (a) was necessary to reduce the concentration of undesirable background material. Specimens were examined in a modified model EM 2/1 microscope made by Metropolitan-Vickers, Ltd., England.

RESULTS

Of the bacteriophages we have examined, those of 9 of the 10 strains of *S. cremoris* and that of the single strain of *S. lactis* are morphologically similar to one another and to those described by Parmelee *et al.* Their sizes all fall within the range given by these authors—heads 60 to 90 mμ in diameter and tails 20 to 40 mμ wide and 120 to 190 mμ long. Type r_1 is typical of this class and is shown in figure 1. The eleventh strain, type d_4, is shown in figure 2 at the same magnification. It is distinctly different in size and shape. Particularly obvious are the larger spherical head and the long slender flagellumlike tail.

The head appears to consist of a sac filled with dense material opaque to the electron beam. Empty head sacs have been seen in whey concentrates of this

Figure 1. Type r_1 bacteriophage. Magnification × 31,000.

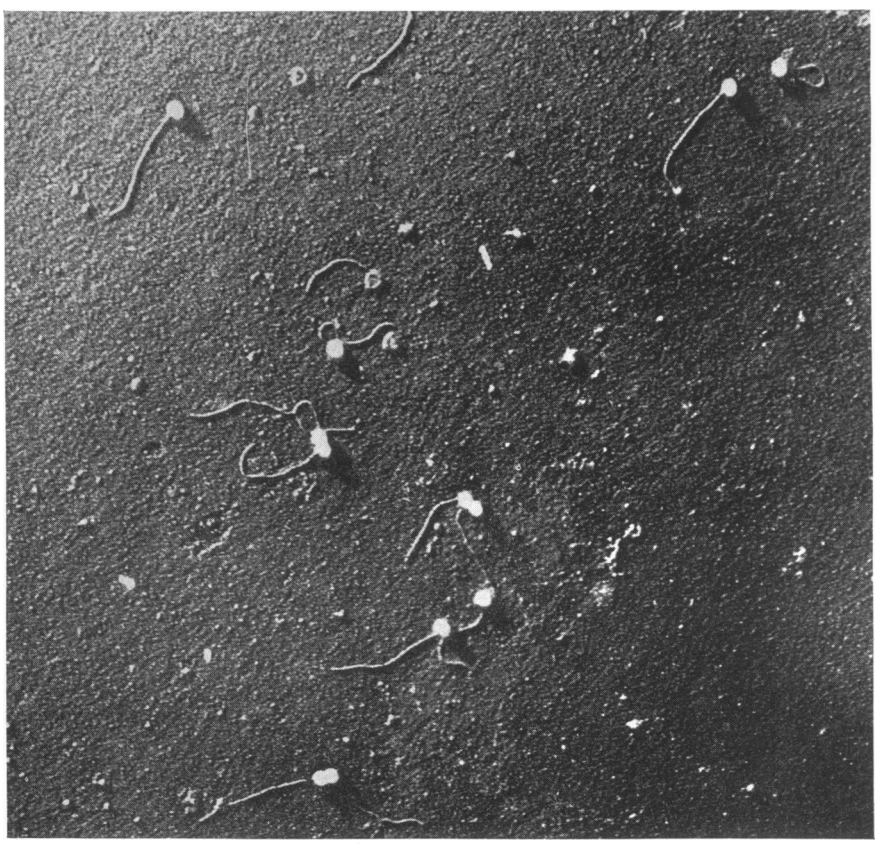

Figure 2. Type d_4 bacteriophage. Note the three empty head sacs in the upper center of the figure. Magnification × 31,000.

type and (less frequently) of the smaller type. Occasionally they appear to have tails attached to them. This feature can be seen in figure 2. They appear also in studies of adsorption of bacteriophages to host cells. In these empty sacs there is seen often a small dense dot suggestive of a central nucleus in the complete bacteriophage. The diameter of the head lies between 70 to 80 mμ.

The most striking feature of the d_4 bacteriophage is its long tail. This is uniform in width and appears to be simply a single fibril. It is 15 mμ wide. The tail, which is apparently very flexible, is seldom straight but usually lies in a curved position making length measurements difficult. The values obtained ranged from 560 to 610 mμ. In figure 2 there appears to be a regular periodic structure of 15-mμ spacing along each tail. Gold, the shadowing metal in these specimens, is known (Williams and Backus, 1949) to agglomerate and produce artifacts in specimens viewed in a microscope fitted with a self-biased gun, but is satisfactory for use with microscopes fitted with a simple gun. At the time when these micrographs were taken, our microscope was fitted with a simple electron gun, and in view of this and the regularity of the spacing, even on curved tails, it is considered that this periodic spacing is a real feature and is not an artifact.

The over-all length of type d_4 bacteriophage is 630 to 690 mμ. This exceeds that of any bacteriophage so far reported.

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SUMMARY

Ten races of bacteriophage active against strains of *Streptococcus cremoris* and one race active against *Streptococcus lactis* have been examined in an electron microscope. Nine of the first group and the one in the second group are morphologically similar to one another and to those reported by others as active against *S. lactis*. The remaining strain is different in size and shape. It has a spherical head, 70 to 80 mμ in diameter, consisting of a cell wall filled with opaque material. This is attached to a long flagellumlike tail 15 mμ wide and 560 to 610 mμ long that is a single fibril. The over-all length exceeds that of bacteriophages so far reported.

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