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Bifidobacterium bacteriophage from calf rumen

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YOUSSEF (1966) first isolated a bifidus specific phage from faeces of sucklings, but did not report about its morphology. Various investigators (SCARDOVI *et al.* 1969, MITSUOKA 1969) have recently identified new species of bifidobacteria in rumen and in faeces of different animals, but phages of these new species are so far unknown.

Several morphological types of phages have been found in both sheep (HOOGENRAAD *et al.* 1967) and bovine (ADAMS *et al.* 1966, BRAILSFORD and HART-

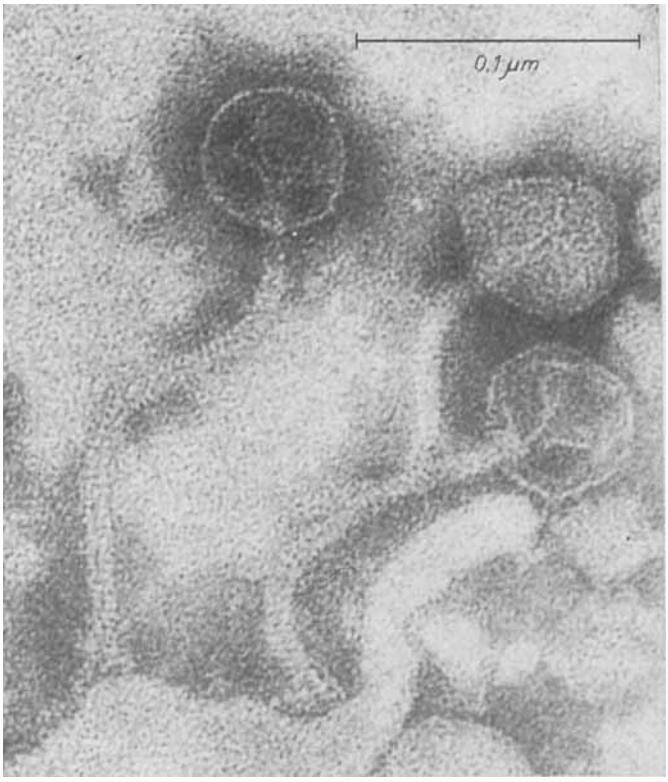

Fig. 1. Phage of *Bifidobacterium ruminale*. Electron micrograph

MAN 1968, PAYNTER *et al.* 1969) rumen contents, but, of the phages isolated from the bovine rumen, only those parasitizing *Streptococcus durans* have been described morphologically (BRAILSFORD and HARTMAN 1968).

In the present note we report the isolation of a phage specific for *Bifidobacterium ruminale*, a bifid bacterium abundant in the rumen of animals fed on diets rich in carbohydrates. The bacterial strain RU 271 was grown in a medium of the following composition: trypticase (BBL) 1.0 g, phytone (BBL) 0.5 g, glucose 1.5 g, yeast extract (DIFCO) 0.25 g, magnesium chloride 0.05 g, zinc sulphate 0.025 g, traces of ferric chloride, distilled water 100 ml.

The cultures were incubated anaerobically at 37 °C in an atmosphere of carbon dioxide under reduced pressure (0.5 atmosphere). The following procedure was used for obtaining the phage: every four days during a 20-days period two litres of liquid culture of *Bifidobacterium ruminale* RU 271 were introduced into the calf rumen by means of a stomach tube. After this period about 50 ml of the rumen content were removed using again a stomach tube. The sample was centrifuged for 5 min at 10,000 rpm and the supernatant freed from the bacteria by membrane filtration under pressure. The sterile filtrate was added to a culture of RU 271 in the exponential growth phase (after 8—10 hours of incubation) to a concentration of 1%. The enrichment technique described by ADAMS (1959) was then applied, resulting in a very concentrated suspension of phage. 1% of this suspension, when added to a culture of RU 271 in the logarithmic growth phase, caused complete clearing within 6—8 hours.

Electronmicroscopic observation of negatively stained specimens confirmed the presence of phage particles, as can be seen from Figure 1. All the phages possess octahedral heads 600 Å in diameter; the tails are 2150 Å long and 110 Å in diameter with a base plate. A regular transversal striation is obvious in all examined preparations. The tails are always slightly curved.

References

ADAMS, M. H., 1959. Bacteriophages, p. 450. Interscience Publishers, Inc., New York.

ADAMS, J. C., GAZAWAY, JR., J. A., BRAILSFORD, M. D., HARTMAN, P. A. and JACOBSON, N. L., 1966. Isolation of bacteriophages from the bovine rumen. Experientia (Basel), **22**, 717.

BRAILSFORD, M. D. and HARTMAN, P. A., 1968. Characterization of *Streptococcus durans* bacteriophages. Canad. J. Microbiol., **14**, 397.

HOOGENRAAD, N. J., HIRD, F. J. R., HOLMES, I. and MILLIS, N. F., 1967. Bacteriophages in rumen contents of sheep. J. gen. Virol., **1**, 575.

MITSUOKA, T., 1969. Vergleichende Untersuchungen über die Bifidobakterien aus dem Verdauungstrakt von Menschen und Tieren. Zbl. Bakteriol., Abt. I, Orig., **210**, 52.

PAYNTER, M. J. B., EWERT, D. L. and CHALUFA, W., 1969. Some morphological types of bacteriophages in bovine rumen contents. Appl. Microbiol., **18**, 942.

SCARDOVI, V., TROVATELLI, L. D., CROCIANI, F. and SGORBATI, B., 1969. Bifid bacteria in bovine rumen. New species of the genus *Bifidobacterium*: *B. globosum n. sp.* and *B. ruminale n. sp.* Arch. Mikrobiol., **68**, 278.

YOUSSEF, M., MÜLLER-BEUTHOW, W. and HAENEL, H., 1966. Isolierung von Bakteriophagen für anaerobe Laktobazillen aus dem Stuhl des Menschen. Naturwissenschaften, **53**, 589.

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