Isolation and Characterization of *Streptococcus agalactiae* Bacteriophage

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Temperate and virulent phages are known to occur in streptococci of groups A, C and D (Bradley & Kay, 1960; Brailsford & Hartman, 1968; Brock, 1964; Follett, 1967; Kjems, 1960; Rogers & Sarles, 1963). No phage has been reported for group B streptococci; we report the isolation of such a phage and give some of its properties.

Cultures of streptococci were isolated from cow's milk and identified as strains of *Streptococcus agalactiae* serologically and by the CAMP test (Christie, Atkins & Munch-Peterson, 1944). Phage suspensions were obtained from 16 hr culture of *S. agalactiae* by centrifugation in a model L Spinco centrifuge (5000 g) for 15 min. followed by filtration of the supernatant fluid through a 0.45 μm pore-size membrane filter (Millipore Corp., Bedford, Mass, U.S.A.). A drop of the filtrate was put on an agar plate seeded with test organisms. Phage activity was indicated by clear plaques after incubation. Phage stocks were plaque-purified and stored at 4 °. Phages were assayed using the soft-agar method (Adams, 1959) and gave clear plaques (0.5 to 1 mm. diam.) which remained clearly visible even after prolonged incubation.

High-titre phage stocks were produced by confluent lysis on agar plates (Adams, 1959). Phage suspensions were prepared by adding 40 ml. phosphate buffered saline (pH 7.4) for 3 to 5 hr. The liquid portion was decanted from the surface of the agar and centrifuged (5000 g) for 30 min. The supernatant fluid was filtered through a membrane filter (0.45 μm) and centrifuged (58,000 g) for 2 hr to sediment the phage particles. The sediment was suspended in buffered saline to about one-sixth the original volume. To avoid inactivating the phage, the pellet was allowed to disintegrate by standing overnight at 4 °. The resulting suspension was again centrifuged (5,000 g) for 30 min. and the supernatant fluid layered on a formed gradient of CsCl specific gravity 1.13 to 1.65. The gradient was made in 28 ml. Oak Ridge type 30 B polycarbonate tubes (International Equipment Co., Needham Heights, Mass.) using a gradient-maker (Britten & Roberts, 1960) modified by Martin & Ames (1961). After centrifuging (55,000 g) for 4 hr visible bands were withdrawn from the top with a cannula and syringe; phage suspensions were dialysed against buffered saline overnight. The banding density was determined by weighing a 20 μl. pipette containing the phage and obtaining the ratio of its weight to that of an equal volume of buffer (R. Wu, Biochemistry Dept., Cornell University, personal communication). The phage appeared in a sharp visible band (Pl. 1) at a density of 1.52 g./cm³. Other bands were visible but no phage was found in them. Phage purity was determined by immunodiffusion tests. Antisera were prepared by inoculating rabbits intravenously with about 10⁶ p.f.u. and subsequently with seven doses each of about 10¹⁰ p.f.u. administered twice weekly and then bleeding the rabbits 5 days after the last injection (Maramorosch & Kopprowski, 1967). Only one line could be detected between rabbit anti-phage sera and purified suspensions of phage.
The nature of the phage nucleic acid was determined by staining with acridine orange and confirmed by enzymic digestion (DNase and RNase, Worthington Biochemical Corp. Freehold, N.J.; Bradley, 1966). The phage suspensions stained green with acridine orange and treatment with MoO₄ did not alter the colour. DNase diminished the intensity of staining; RNase had no effect. The results indicate that the phage contains double-stranded DNA.

The phage was sensitive to slow freezing at -20 ° and to heating at 60 °, 1 hr. Chloroform at a final dilution of 1/20 inhibited phage infectivity. The phage could be stored in buffered saline or brain–heart infusion broth at 4 ° without loss of infectivity.

The morphology of the phage was examined in a Hitachi model HU-11 electron microscope at 75 kv. Negatively stained preparations were made by placing a drop of phage suspensions on a formvar-carbon coated grid, removing the excess fluid with a piece of filter paper and allowing to dry thoroughly. A drop of 2 % phosphotungstic acid adjusted to pH 6.0 was added, excess removed and the grid allowed to dry. The grid was immediately examined. The phage particles had relatively small heads, many of which were hexagonal in outlines (Pl. 2a). Of 25 measured, the mean size was 614 Å long (range 534 to 653 Å) by 564 Å wide (range 445 to 594 Å). The tails appeared flexible and non contractile, with a diameter of about 75 Å; it was marked with fine striations with a periodicity of 30 Å.

This investigation has shown that lysogeny exists in Lancefield’s group B streptococci. The initial phage isolates were obtained from broth cultures of Streptococcusagalactiae. This was possible because bacterial cells of some lysogenic strains undergo spontaneous lysis on cultivation, and liberate phage particles into the media.

Morphologically, the Streptococcusagalactiae phages observed seem to fit Bradley’s class B bacteriophages (Bradley, 1967). The small heads appear to have icosahedral symmetry. The fine striations of the tail may indicate helical fine structure. Terminal knobs or tail plates were not observed in the preparations examined.

We agree with Follett (1967) that there appears to be considerable diversity of structure among phages active against the genus Streptococcus. The S. faecalis phages studied by Rogers & Sarles (1963) have elongated heads and knobs attached to the tails, whereas those studied by Follett (1967) have no elongated head but tail knobs are present. Phages active against S. durans (Brailsford & Hartman, 1968) have tail knobs but no elongated heads. The phages active against S. agalactiae do not compare morphologically with any of the above mentioned since they have neither elongated heads or tail knobs.

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Banding of *S. agalactiae* phage (arrow) in a caesium chloride gradient after centrifugation at 55,000 g for 4 hr.

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(Facing p. 316)
(a) Electron micrograph showing negatively stained preparation of *S. agalactiae* phage.

(b) Electron micrograph showing a portion of the tail of a *S. agalactiae* phage particle exhibiting periodic striations.

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Short communications

REFERENCES


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