THE ISOLATION OF A BACTERIOPHAGE ACTIVE AGAINST CORYNEBACTERIUM PSEUDOTUBERCULOSIS

Dexter H. Howard and G. J. Jann
Department of Bacteriology, University of California, Los Angeles, California

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Bacteriophages active against Corynebacterium diphtheriae have been detected and are being studied with increasing frequency (Groman and Lockart, J. Bacteriol., 66, 178, 1953; Barksdale and Pappenheimer, J. Bacteriol., 67, 220, 1954). Recently, bacteriophage activity has been reported in another group of the human corynebacteria, the diphtheria-like corynebacteria (Howard and Jann, J. Bacteriol., 68, 316, 1954). The present note is a report of the isolation and preliminary characterization of a bacteriophage lytic for one of the animal corynebacteria, C. pseudotuberculosis.

The technique used in the detection of the phage was that originally employed by Warner (Brit. J. Exptl. Pathol., 31, 112, 1950) for the detection of phage from lysogenic cultures of Pseudomonas pyocyanea. Other procedures employed in the further characterization of this phage have been described previously (Howard and Jann, J. Bacteriol., 68, 316, 1954).

The 8 strains of C. pseudotuberculosis used in this study were examined and found to be in morphological and biochemical accordance with the accepted criteria for this species (Breed, Murray, and Hitchens, Bergey's Manual of Determinative Bacteriology, 6th ed., 1948, The Williams and Wilkins Co., Baltimore, Md.). The 8 cultures were received through the courtesy of Dr. R. A. Packer, Iowa State College, Ames, Iowa, and Dr. H. R. Carne, University of Sydney, Sydney, Australia.

Employing the Warner technique, one susceptible host (Ov5) was identified. Since the original pool of organisms from which this phage was derived consisted of 8 members, it was not possible to identify the lysogenic source or to know whether there was more than one phage involved. In order to elucidate these two problems, mixed broth cultures were prepared of the indicated host (Ov5) and each of the remaining constituent organisms of the original pool. After 24 hours' incubation at 37°C, these mixed cultures were centrifuged at 2,500 rpm for 30 minutes and the supernate passed to separate 2 hour cultures of the host (Ov5). These cultures were incubated for 24 hours at 37°C and then centrifuged at 2,500 rpm for 30 minutes. The supernates were spotted on agar overlay plates (Adams, Methods in Medical Research, Vol. 2, 1950) of Ov5. The results indicated that the phage active against Ov5 came from a single lysogenic source, strain Ov3.

Strain Ov5 is the only strain of C. pseudotuberculosis found to be sensitive to the phage. The phage did not attack other corynebacteria tested including 27 strains of C. diphtheriae, 3 of C. xerose, 2 of C. pseudodiphtheriticum, 24 of diphtheria-like corynebacteria, 7 of C. renale, and 4 of C. equi.

FURTHER OBSERVATIONS ON THE BACTERIAL SPORE NUCLEUS

M. E. Hunter and E. D. DeLamater
Section on Cytology and Cytochemistry, School of Medicine, University of Pennsylvania, Philadelphia, Pennsylvania

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Previous observations of the authors on the nuclear cytology of the spore of Bacillus megaterium (DeLamater and Hunter, J. Bacteriol., 63, 13, 1952; Hunter and DeLamater, J. Bacteriol., 63, 23, 1952) were in contradiction to certain views of Robinow (J. Gen. Microbiol., 5, 439, 1951). While Robinow considered the spore nucleus in hydrolyzed and stained preparations to
be located peripherally in the spore, DeLamater and Hunter believed it to be centrally placed. Since it has been more recently demonstrated that extrusion of the spore nucleus may be produced by the acid hydrolysis step in the usual nuclear staining methods for bacteria (Bisset and Hale, J. Hyg., 49, 201, 1951; Robinow, J. Bacteriol., 66, 300, 1953; Fitz-James, J. Bacteriol., 66, 312, 1953), the above discrepancy is no longer of the same basic interest. Because clarification seemed desirable, a study was undertaken to determine the cause of the discord. It does not appear to lie in method, nor in confusion of germinating spores with resting spores (Robinow, J. Bacteriol., 65, 378, 1953). Observations made on spores taken from cultures grown on different media indicate that the composition of the substrate is of importance. Spores taken from cultures grown on a special nutrient agar (Difco, Control no. 383273; 23 g per liter H₂O), on which sporulation was almost 100 per cent in 24 hours, showed 100 per cent peripheral nuclei when hydrolyzed and stained in thionin-SO₂ (DeLamater, Stain Technol., 26, 199, 1951), with the exception of a small percentage which were in the process of germination. On the medium previously used by DeLamater and Hunter (0.1 per cent casamino acids (Difco); 0.5 per cent NaCl; 1.5 per cent agar; adjusted to pH 7) the nuclei again appeared to be centrally located in almost 100 per cent of spores. Unhydrolyzed spores from both media were highly refractile and failed to stain with methylene blue.

Bisset and Hale (J. Hyg., 49, 201, 1951) showed that the degree of displacement of nuclear material in bacterial spores varies with the species. Keigler and Smith (J. Histol. Cytochem., 2, 233, 1954) found that spores of Bacillus cereus grown on a dilute casein hydrolyzate medium showed both a central and a peripheral chromatic body. Very minute basophilic granules sometimes appear at the periphery of spores of B. megaterium formed on the 0.1 per cent casein hydrolyzate medium, the significance of which is not yet clear. These observations indicate that the extent of displacement of nuclear material in spores by acid hydrolysis is influenced by the composition of the medium upon which the culture was grown, and that in the case of B. megaterium it is almost nil when grown on 0.1 per cent casein hydrolyzate.

Figure 1. Bacillus megaterium spores from a sporulating culture 48 hours old on dilute casein hydrolyzate agar. Fixed in OsO₄ vapors, hydrolyzed in 1 N HCl at 60 C for 5 min, stained in 0.25 per cent thionin containing 0.1 per cent thionyl chloride, frozen-dehydrated in absolute ethyl alcohol.

Figure 2. Bacillus megaterium spores from a sporulating culture 48 hours old on a special nutrient agar (Difco, Control no. 383273). Same staining procedure.