Influence of bacteriological media constituents on the reproduction of *Salmonella enteritidis* bacteriophages

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Three different *Salmonella enteritidis* phages were isolated and purified from raw sewage by agar-layer technique. The sensitivity of the host organisms toward phages was changed when they were grown on different bacteriological media.

The effect of single components of the medium on phage reproduction was determined by the omission of that substance from the medium. CaCl\(_2\), MgSO\(_4\), and glycerol each had a pronounced stimulatory effect on the phage reproduction, while bile salts had a profound inhibitory effect. The inhibitory effect of bile salts on phage growth was much greater on one strain of *Salmonella enteritidis* than on the other.

**INTRODUCTION**

Various chemical agents are incorporated in different bacteriological media to inhibit the growth of some bacteria while enhancing the growth of others. Some of these media constituents have been reported to affect the growth of some bacteriophages (Schultz and Krueger, 1928; Cherry and Watson, 1949; Weissbach and Jacob, 1962; Shafia and Thompson, 1964).

Present investigation was undertaken to determine whether different bacteriological media used to grow the host organism (*Salmonella enteritidis*) would influence the phage multiplication; and if so, which media constituent(s) would be responsible for the observed effects.

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MATERIALS AND METHODS

Host organisms. Two different strains of Salmonella enteritidis 1891 from the Department of Botany and Bacteriology, University of Arkansas, were used as the host organisms. They have been designated as S. enteritidis A, a relatively avirulent strain, and S. enteritidis B, a virulent strain. The strains gave identical biochemical and serological reactions signifying their type identity.

Isolation of S. enteritidis bacteriophages, preparation of phage stocks, and method of assays. Untreated sewage was obtained from the local municipal sewage disposal system. One-hundred-ml aliquots were centrifuged at 3000 × g for 15 min; the supernatant was collected and refrigerated. The host organism was prepared by inoculation of 100 ml of tryptose broth (Difco) with 1 ml of 5-h culture of S. enteritidis A. Five ml of prepared centrifuged sewage was added immediately and thoroughly mixed. After 24 h incubation at 37 C, the culture was centrifuged at 3000 × g for 10 min to remove bacterial debris. The supernatant was then filtered through an ultratine glass filter (Morton Bacterial Pyrex Brand 9-754) and examined for the presence of phage using soft agar overlay technique (Adams and Swanstrom, 1951).

Serial 10-fold dilutions of phage suspensions were made in isotonic saline (pH 6.9 to 7.1), and 1-ml quantities of those in the appropriate range were added to the tubes containing 2.5 ml of melted, soft agar (0.7% at 46 C) which had been seeded with 0.2 ml of the 5-h culture of the host organisms immediately before addition of the phage. After thorough mixing, the contents of each tube of soft agar were quickly poured over a tryptose agar plate (containing 20 ml of 1.4% agar). The plates were incubated at 37 C for 24 h. Three types of plaques were recovered by this method. A single plaque of each type was picked by a sterile transfer loop and rinsed in 1 ml of sterile broth. Several dilutions of each broth were made and plated as above. This procedure was repeated four times in order to obtain a pure culture of each plaque type.

High-titer phage stocks of these three phages were prepared by transferring about 40 identical plaques from the same plate into the fresh culture of the S. enteritidis A. The mixture was incubated at 37 C until lysis of bacteria occurred and the culture became clear. The lysates were filtered and assayed by soft agar overlay technique. Triplicate plates were poured for each phage dilution and plates with 30–100 plaques were selected for the final counts. Each phage was designated with a serial number (phage no. 1, no. 2, and no. 3) and filtrates were dispensed aseptically in 5-ml quantities into screw-capped tubes and stored at −20 C.