JOURNAL OF VIROLOGY, Mar. 1969, p. 350

Vol. 3, No. 3
Printed in U.S.A.

NOTES

Isolation of Bacteriophage for *Clostridium tetani*

DIANE ROSEMAN AND ROBERT L. RICHARDSON
Department of Microbiology, University of Iowa, Iowa City, Iowa 52240

Received for publication 27 January 1969

The purpose of this report is to note the isolation of a *Clostridium tetani* phage from soil and to specify the procedure used to obtain suitable lawns for plaque formation.

There is, to our knowledge, one report of the isolation of a *Clostridium tetani* bacteriophage (1). Details of the methods used in growing the phage to observe plaque formation were not presented in that report. The existence of *C. tetani* phages was confirmed with the publication of electron microscope photographs showing phages in pellets obtained from mitomycin C-induced lysates of *C. tetani* cultures (2).

In the present study, a garden soil sample (20 g) was inoculated into 1.5 liters of tryptic soy-cysteine broth contained in a 2-liter flask. The culture was incubated for 36 hr at 37 C in air. The supernatant fluid was collected, passed through a sterile membrane filter (0.45 μm), and tested for phage by plaque formation on several *C. tetani* strains. On the lawn of one strain, plaques were observed. After three successive platings from isolated plaques, a lawn which showed almost confluent lysis was washed with sterile broth. This broth was collected and filtered, and 0.5 ml was inoculated into a young broth culture of the indicator strain. The culture became clear after 3 hr, and the lysate was passed through a sterile filter. The filtered lysate was used to determine the susceptibility of 26 *C. tetani* strains to the phage by plaque formation.

Two factors were particularly important in preparing double-layer agar plates with suitable lawns for plaque studies. First, it was important that the bottom-layer agar was not too moist; second, it was necessary that cells from young broth cultures be concentrated to provide confluent lawns. The medium for the bottom layer was tryptic soy broth (Difco) plus 0.05% cysteine hydrochloride and 1.5% Ionagar no. 2 (Colab). Approximately 40 ml of medium was poured in a petri plate. After the agar solidified, the glass petri lids were replaced with sterile unglazed procelain lids, and the plates were incubated anaerobically (90% H_2 and 10% CO_2) in Torbal jars for 12 hr at 37 C. After incubation, the top layer was poured. Top-layer medium was identical to the bottom-layer medium, except that 0.35% Ionagar was used and $CaCl_2$ (11 mg/liter) was added. To 2.5 ml of melted top-layer agar were added 1.0 ml of a concentrated suspension of *C. tetani* and 0.25 ml of filtered lysate. The concentrated *C. tetani* suspension was prepared by resuspending the pellet obtained from 10 ml of a 10-hr culture in 1.2 ml of sterile broth. After the top layer was poured, the plates were covered with sterile glass lids, incubated anaerobically at 37 C for 18 hr, and observed for plaques.

The phage that was isolated produced plaques on 11 of 26 toxin-producing *C. tetani* strains. Plaques averaged 1.0 mm in diameter. Young broth cultures of plaque-susceptible strains became clear 2 to 5 hr after addition of phage (phage to bacteria ratio, 10 to 1). Filtered lysate preparations usually had plaque-forming titers of 10^8 or higher. Electron microscope photographs of the phage showed that it has a head and tail.

LITERATURE CITED

1. Cowles, P. B. 1934. A bacteriophage for Cl. tetani. J. Bacteriol. 27:163–164.
2. Prescott, L. M., and R. A. Altenbern. 1967. Detection of bacteriophages from two strains of *Clostridium tetani*. J. Virol. 1:1085–1086.