JOURNAL OF BACTERIOLOGY, Sept., 1966
Vol. 92, No. 3

Use of Tetrazolium for Improved Resolution of Bacteriophage Plaques

P. A. PATTEE
Department of Bacteriology, Iowa State University, Ames, Iowa

Received for publication 12 May 1966

Most phages form plaques which, when prepared by the agar-layer technique (M. H. Adams, *Bacteriophages*, Interscience Publishers, Inc., New York, 1959), are sufficiently pronounced to permit their accurate enumeration under appropriate conditions of illumination. Other phages, however, are difficult to detect and enumerate because their plaques are small or excessively turbid, or both. Phage 83, employed for the genetic analysis of *Staphylococcus aureus* (W. E. Kloos and P. A. Pattee, J. Gen. Microbiol. **39**:195, 1965), and the majority of other phages active against this species, form plaques of highly variable, small size. This report is concerned with a rapid and simple modification of the plaque assay technique which greatly facilitates the assay of staphylococcal phages, and which should have considerable application in the study of other phages.

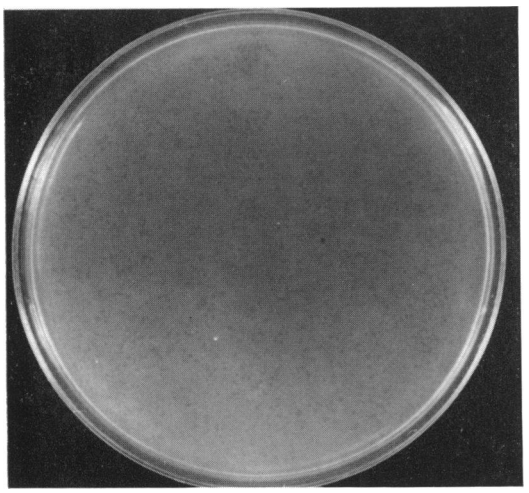

FIG. 1. *Appearance of plaques of phage 83 after incubation of assay plate for 8 hr at 37 C. (Photographed by oblique illumination against a black background.)*

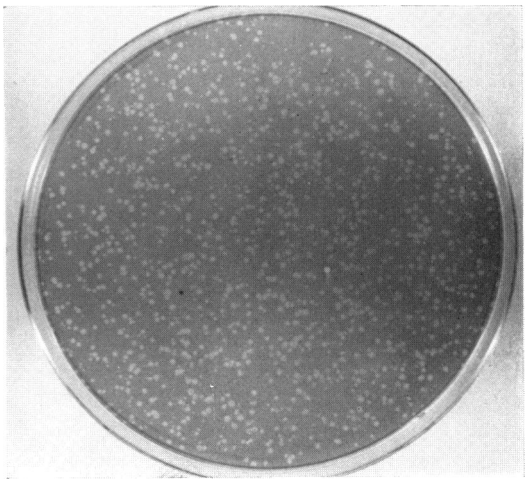

FIG. 2. *Appearance of plaques shown in Fig. 1 after exposure to 0.1% triphenyltetrazolium chloride for 20 min at 37 C. (Photographed by oblique illumination against a white background.)*

The agar-layer technique for the plaque assay of staphylococcal phage 83 was conducted in the usual manner with *S. aureus* Ps 83, P and D agar, and P and D soft agar (P. A. Pattee and J. N. Baldwin, J. Bacteriol. **82**:875, 1961). After incubation of the assay plates for 8 hr at 37 C, the plaques were sufficiently developed to be scored (Fig. 1). The assay plates containing the fully developed plaques were then flooded with 10 ml of Trypticase Soy Broth (TSB, BBL) containing 0.1% 2,3,5,-triphenyltetrazolium chloride (TTC, Nutritional Biochemicals Corp., Cleveland, Ohio). After incubation at 37 C for 20 min, the broth was poured off. Each plaque was now a sharp, clear area against the intense red background produced by the reduction of TTC to the insoluble formazan by the indicator cells (Fig. 2). In addition to TSB, distilled water, 0.5% NaCl, 1% glucose, and P and D broth were examined as suspending media for TTC. Although identical results were eventually obtained in all cases, only P and D broth supported the reduction of TTC at a rate similar to that obtained with TSB. Similarly, incubation of the flooded plates at 37 C merely accelerated the rate at which TTC was reduced.

Plaques formed by the T-series of coliphages, and phages M13, P22, and λ have been examined by this technique, with the use of nutrient agar and soft agar containing 0.5% NaCl. In all instances, TTC was reduced, but with the exceptions noted below little advantage was gained, owing to the resolution of the unstained plaques. The inherent variations in plaque morphology with phage P22 were enhanced after TTC treatment. This was not true in comparing *r*+ and *r* plaques of phage T4. Phage M13 forms very turbid plaques whose resolution was vastly improved after TTC treatment. When old (ca. 18 to 20 hr) plaques of phage M13 were treated with TTC, a peripheral staining effect was obtained which is attributed to the lessened activity of the indicator cells in reducing TTC, and the enhanced permeability of infected cells which are producing progeny phage (W. O. Salivar et al., Virology **24:** 359, 1964).

Triphenyltetrazolium chloride is being employed routinely in this laboratory as an aid in scoring plaques formed by staphylococcal phages, a practice which has increased the accuracy of the titration procedure and has reduced significantly the time required to score plaques. This technique should prove useful in the search for new phages, many of which may have been overlooked in the past because of the nature of their plaques. In addition, the technique may be of value in the study of plaque-morphology mutants of phage.

This study was supported in part by grant GB-2325 from the National Science Foundation and Public Health Service grant AI-06647 from the National Institute of Allergy and Infectious Diseases.