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A Research Note

Simplified, Rapid Method to Measure Diameter of Bacteriophage Plaques[1]

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ABSTRACT

A procedure is described for accurately determining the diameter of bacteriophage plaques down to .05 mm in diameter.

Different phage races may form plaques of different size and shape (*2*), and growth conditions also influence the diameter of bacteriophage plaques (*4*). Potter and Nelson (*3*) relied upon the number and size of plaques to judge the stimulatory or inhibitory effect of minerals on plaque formation by lactic streptococci bacteriophages.

The diameter of bacteriophage plaques can be measured roughly by using a ruler (*1*). This procedure is not considered accurate, however, and effective comparison between plaques formed under different conditions is difficult. With a ruler, reasonable measurement precision could be obtained only at the millimeter level. A preferred and more precise method of measuring plaques is by microscopy (*1,3*). Drawbacks of this method are: (*a*) the objective lens of the microscope sometimes touches the agar overlay and may disturb the contour of the plaque and cause error, (*b*) sometimes plaques are hard to view microscopically because of illumination problems, (*c*) unless the agar overlay is uniform, it is very difficult to obtain accurate plaque measurement, and (*d*) the method is tedious and time consuming especially when a number of plaques are to be measured.

MATERIALS AND METHODS

A simplified and dependable technique for measuring plaque size has been developed that used the appliances shown in Fig. 1. The upper left hand side of the figure shows a micrometer (Bausch and Lomb, Inc., Rochester, New York) which is equipped with a removable battery-powered illumination device. In the upper right-hand of the figure is a calibration disc which is calibrated in millimeters. The calibration disc is used to check the micrometer divisions. Below the micrometer and calibration disc is a divisor. The equipment is placed on white blotting paper.

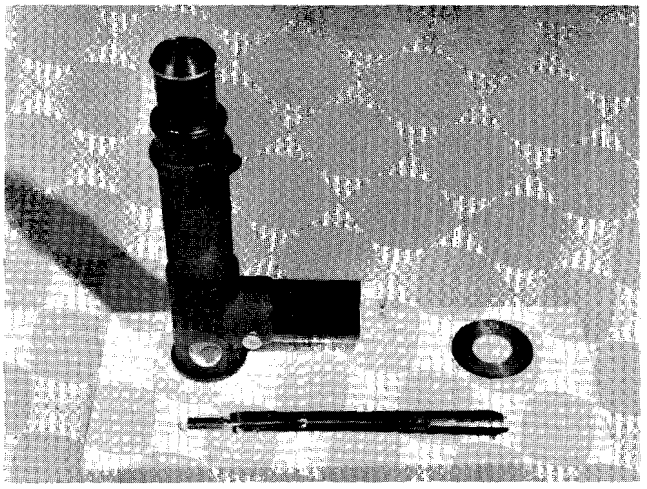

Figure 1. *Apparatus used to measure the diameter of* S. thermophilus *bacteriophage plaques. Micrometer equipped with battery illumination device, divisor, and blotting paper.*

The detailed procedure for determining plaque diameters is as follows: (*a*) the divisor is adjusted to the size of the plaque, (*b*) impressions are made on the underlying blotting paper by pressing gently with the divisor, (*c*) the micrometer is placed on the blotting paper in such a way that the impressions fall in the middle of the open area of the micrometer, (*d*) the magnified centers of the impressions are matched with the calibrated scale of the micrometer and the diameter is read directly to one-hundredth of a millimeter. For minimum error, diameters of ten contiguous plaques on duplicate plates are determined, and the diameter of the 20 plaques are averaged.

RESULTS AND DISCUSSION

With this procedure, plaque diameters of *Streptococcus thermophilus* bacteriophage were determined under various growth conditions, and excellent results

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were obtained throughout the investigation. Advantages of this method over others are (*a*) ease and simplicity, (*b*) diameters of plaques situated at the periphery of a plate are easily measured, (*c*) even if the agar overlay is irregular, error is minimal, and (*d*) rapidity.

The procedure described in this paper could also be used successfully to measure the size of bacterial colonies.

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