APPLIED MICROBIOLOGY, Jan. 1974, p. 290-291
Vol. 27, No. 1

Comparison of Methods for Enumerating Fluorescent Bacteria[1]

W. F. McTERNAN, J. C. ADAMS, AND P. A. RECHARD

Division of Microbiology and Veterinary Medicine, and Water Resources Research Institute, University of Wyoming, Laramie, Wyoming 82070

Received for publication 21 August 1973

Comparable numbers of fluorescent bacteria may be obtained by either the most-probable-number procedure or by preparing spread plates on modified Henrici agar.

Because "ecology" and "eutrophication" are words that have come into vogue, there is growing concern about the addition of nutrients to water. The commonly accepted bacterial indicators of pollution, the total coliforms, fecal coliforms, fecal streptococci, and the standard plate count at 35 C, are more concerned with public health aspects of pollution than they are with the addition of nutrients to water. The fluorescent pseudomonads have been proposed as a group of bacteria that may respond to surface runoff from agricultural lands by increasing in numbers (2). Additionally, one component organism of the group, *Pseudomonas aeruginosa*, might also be an indication of fecal pollution (1, 2). Moreover, the pseudomonads might exhibit a decrease in a stream (2), as a function of time; thus, their numbers might indicate how recent the addition of sewage or runoff was. In contrast, this group of organisms has been termed an indigenous component of the bacterial stream flora, which responds to pollution by increasing in numbers (D. L. Johnstone, Ph.D. thesis, Washington State Univ., Pullman, Wash., 1970). Because the most-probable-number (MPN) technique has been recommended for the determination of these organisms (2) and because it is a tedious procedure, the present study was initiated to determine if a simpler method could be used to monitor this group of organisms.

Water samples were collected aseptically from various streams in Wyoming in sterile wide-mouth 1-liter polypropylene bottles, iced, and returned to the laboratory for analysis. Samples were processed within 4 to 8 h after collection, depending upon travel time back to the laboratory. Each sample was diluted, spread plates were made in duplicate at the appropriate dilutions on modified Henrici agar (3), and they were incubated at 20 C for 7 days. The formulation of modified Henrici agar is: peptone, 0.5 g; glycerine, 0.5 ml; soluble starch, 0.5 g; sodium caseinate, 0.5 g; K_2HPO_4, 0.3 g; $MgSO_4$, 0.2 g; $FeSO_4$, a trace; agar, 15.0 g; and distilled water, 1 liter. The pH is adjusted to 7.4 with NaOH before autoclaving. Additionally, a five-tube MPN determination was run in the basal mineral salts medium of Drake (1) with glycerol deleted. Incubation for the MPN determination was at 20 C for 1 week. In some cases the basal medium was solidified with 1.5% agar, and spread plates were made at appropriate dilutions. Fluorescence was detected by means of a Woods ultraviolet lamp.

The numbers of fluorescent bacteria determined upon modified Henrici agar compared favorably with those determined by the MPN procedure. The MPN procedure yielded counts higher than the modified Henrici plates in 15 of 41 samples, or 37% of the time. In contrast, the fluorescent bacterial counts on modified Henrici agar were greater than the numbers obtained by the MPN method 44% of the time, or in 18 of the 41 samples. In addition, in 8 of the 41 samples or 19% of the time, the counts were essentially the same by both procedures. Only in 3 of 41 samples was there a greater than 10-fold difference in the numbers observed by the two methods. The mean and the standard error of the mean for 41 determinations on modified Henrici agar were 1,333 and 497, respectively, whereas the values for the MPN procedure were 1,050 and 308. A student t test done on the differences between paired samples gave a t value of 0.81 which showed no statistical difference between the two procedures at the 1% confidence level. The correlation coefficient for paired samples was 0.719, which is significant at the 0.001 confidence level for 40° of freedom.

When the basal medium was solidified with agar and spread plates were made, the numbers obtained were similar to those obtained on

[1] Published as journal paper no. 607, with the approval of the Director, Wyoming Agriculture Experiment Station.

modified Henrici agar. However, diffusion of the fluorescent pigments made enumeration after 1 week of incubation quite difficult. This problem was not as prominent on modified Henrici agar. Additionally, incubation of the MPN tubes for periods longer than 1 week did not consistently yield increased counts.

The use of a Woods lamp is mandatory for identifying the fluorescent colonies on the modified Henrici agar plates, and, as Drake (1) points out, facilitates the identification of the positive tubes in the MPN procedure. It is concluded that counts of fluorescent bacteria comparable to the MPN method can be obtained by carefully counting fluorescent colonies on spread plates of modified Henrici agar. The latter procedure has the advantage of mechanically simplifying the enumeration of fluorescent bacteria in natural waters and also allows the enumeration of the total viable aerobic bacteria in the water.

We thank L. R. Maki for statistical analysis, and the American Metal Climax Company and the Bureau of Reclamation for their support in this study.

LITERATURE CITED

1. Drake, C. H. 1966. Evaluation of culture media for the isolation and enumeration of *Pseudomonas aeruginosa*. Health Lab. Sci. **3**:10–19.
2. Hoadley, A. W., and E. McCoy. 1966. Studies of certain gram-negative bacteria from surface waters. Health Lab. Sci. **3**:20–32.
3. Stark, W. H., and E. McCoy. 1938. Distribution of bacteria in certain lakes of northern Wisconsin. Zentralbl. Bakteriol. Parasitenk. Infektionskr. Abt. 2 **98**:201–209.