Membrane Filtration Faecal Coliform Determinations with Unmodified and Modified M-FC Medium

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Abstract

Membrane filtration counts of faecal coliform bacteria did not differ significantly on M-FC medium with and without rosolic acid. The elimination of rosolic acid did not result in an increase in false positive isolates or interfering background growth.

Introduction

Faecal coliform determinations are the major tool in the bacteriological assessment of faecal pollution of aquatic systems. Selective recovery of these bacteria is achieved by using a basal medium containing lactose as the fermentable substrate and selective inhibitory agents coupled with an elevated incubation temperature.

The procedure for the determination of faecal coliform numbers recommended in *Standard Methods* (APHA, 1975) is the membrane faecal coliform method employing the M-FC medium of Geldreich, Clark, Huff and Best (1965) with an elevated incubation temperature of 44,5°C. The M-FC medium contains bile salts, rosolic acid and aniline blue as its selective and indicator agents. The aniline blue/rosolic acid indicator system was investigated by Bronfenbrenner, Schlesinger and Soletsky (1920) who found that the indicator system had bacteriocidal properties towards Gram positive bacteria. They found that this property was due solely to the action of rosolic acid. Geldreich *et al.* (1965) incorporated the sodium salt of rosolic acid into their medium and found that non-faecal bacteria from some water sources were suppressed.

The use of selective media for the isolation of different groups of bacteria is a standard tool in bacteriology. However when using selective inhibitors care is needed to ensure that inhibition of the group of bacteria being isolated does not occur, particularly if they are present in stressed environments, e.g. chlorinated sewage effluents. Ideally a medium should contain no inhibitory agents.

The elimination of rosolic acid from the M-FC medium of Geldreich *et al.* (1965) has been proposed by Presswood and Strong (1978) who reported that faecal coliform counts from chlorinated sewage improved by 49% when using M-FC medium without rosolic acid. Of 200 chlorinated and unchlorinated sewage, lake, creek and river samples 71% had higher counts on M-FC medium without rosolic acid without background overgrowth.

The elimination of rosolic acid would result in cost and labour saving as this constituent is prepared separately prior to addition to the basal medium.

The purpose of this study is to assess the use of modified M-FC medium, i.e. without rosolic acid, on raw sewage, unchlorinated final effluent and river samples under South African conditions.

Materials and Methods

Samples

Raw sewage and final effluent samples were collected from Baviaanspoort sewage works, 20 km North-East of Pretoria, over a 7 month period. River samples were collected at sampling points on the Pienaars River, before and after the outfall of the sewage works, and at sampling points on the Moretelespruit,* the Hartbeesspruit and at Department of Water Affairs weirs on the Hartbeesspruit, Pienaars River and Edendalespruit where they enter Roodeplaat Dam. A sample was also taken from the irrigation channel leaving Roodeplaat Dam. The Moretelespruit, the Hartbeesspruit and the middle reaches of the Pienaars River are subject to agricultural and industrial runoff pollution.

All raw sewage samples and all but one of the final effluent and effluent polluted river samples were analysed within 1 h of collection. All other samples were stored in cool bags with ice immediately after collection and analysed within 6 h.

Analysis of samples

M-FC medium (Difco Laboratories) was prepared on the day of use and after preparation split into two lots. A freshly prepared solution of 1 g dm^{-3} rosolic acid (Riedel-de Haën A-G.) in 0,2 mol.dm^{-3} NaOH was added to one portion. The final concentration of rosolic acid in the medium was 0,01 g dm^{-3}. Rosolic acid was excluded from the second portion.

Samples were analysed by the membrane filter procedure as described in *Standard Methods* (APHA, 1975). Throughout the study Millipore HC membrane filters were used. The filters were incubated at 44,5°C ± 0,2°C for 24 h, after which all typical blue colonies were counted and colonies picked for confirmation.

Confirmation of faecal coliform colonies

Confirmation of blue colonies was done by random isolation of colonies and inoculation into tubes of lactose peptone water with Durham tubes (for gas production from lactose fermentation at 44,5°C) and tryptone water (for indole production at 44,5°C). The tubes were incubated at 44,5°C ± 0,2°C for 24 h. All cultures producing gas from lactose and indole from tryptone water were recorded as faecal coliforms.

Analysis of data

Two-variable linear regression analysis was performed on the data (Steel and Torrie, 1960).

*The Place Name Committee decided in 1963 that the correct name is Moretelespruit and not Moreletaspruit as commonly known. The matter is, however, receiving new attention.

Results

A total of 46 samples were processed and the counts obtained on M-FC medium without rosolic acid were higher than those on M-FC medium with rosolic acid in 56,5% of samples analysed. Lower counts on M-FC medium without rosolic acid were found in 32,6% of samples analysed. A total of 1 171 colonies were picked off for confirmation, 573 from M-FC medium with rosolic acid and 598 from M-FC medium without rosolic acid. Of the 598 colonies from the modified M-FC medium, 548 (91,6%) were confirmed as faecal coliforms, while of the 573 colonies from the M-FC medium with rosolic acid 521 (90,9%) were confirmed as faecal coliforms. Thus the elimination of rosolic acid did not result in an increase in the number of false positive isolates.

A highly significant correlation, indicated by the correlation coefficient $r = 0,99$ ($p = 0,01$), existed between log faecal coliform counts on M-FC medium with rosolic acid and log faecal coliform counts on M-FC medium without rosolic acid (Figure 1), described by the equation $y = 0,99x - 0,02$ where y = M-FC with rosolic acid counts and x = M-FC without rosolic acid counts.

As noted by Presswood and Strong (1978) the counting of the colonies on the M-FC medium without rosolic acid was easier, as the contrast between the blue faecal colonies and the pale cream/brown non-faecal coliform colonies was more pronounced. This improvement was very evident in this study.

Discussion

Standard Methods (APHA, 1975) states that if results indicate that rosolic acid is not required it may be left out of M-FC medium.

Presswood and Strong (1978) showed that the elimination of rosolic acid from M-FC medium improved faecal coliform recovery from chlorinated and unchlorinated sewage and creek, lake and river samples. In their study counts from chlorinated sewage samples improved by 49%. Of 200 samples, counts were higher on M-FC medium without rosolic acid in 71% of samples. In this study, using 46 samples, counts were higher in 56,5% of samples and lower in 32,6% of samples. There was, however, no statistically significant difference ($p = 0,01$) between using M-FC medium with or without 0,01 g dm^{-3} rosolic acid when routine river, raw sewage and final effluent samples were examined. The exclusion of rosolic acid did not result in an increase of false positives or an overcrowding of non-faecal coliform background counts under the conditions of this study.

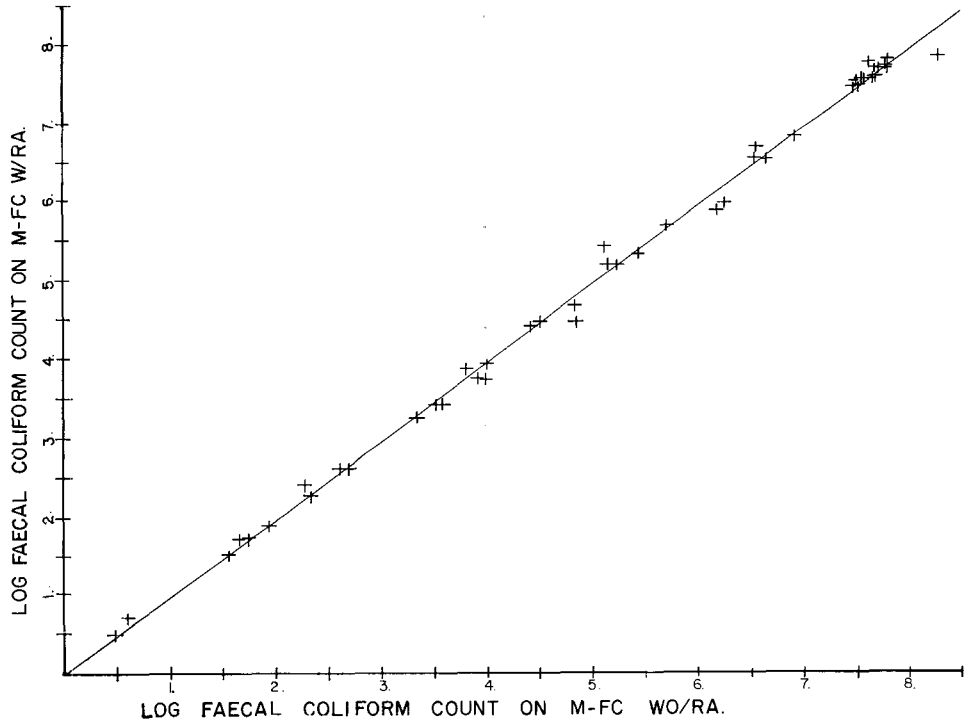

Figure 1
Two-variable linear regression analysis on log faecal coliform counts on M-FC media with rosolic acid (M-FC W/RA) and without rosolic acid (M-FC WO/RA) from raw sewage, final effluent and river samples

Counting of colonies was easier on M-FC medium without rosolic acid due to better colony differentiation and eradication of variable background colour. Thus the elimination of rosolic acid can be safely implemented without impairing the selective quality of the M-FC medium, and with the improvement of recovery of chlorine stressed faecal coliforms. The elimination of rosolic acid is also a cost and labour saving modification.

Acknowledgement

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References

AMERICAN PUBLIC HEALTH ASSOCIATION (1975) Standard methods for the examination of water and wastewater. 14th Edition APHA, New York.

BRONFENBRENNER, J., SCHLESINGER, M.J. and SOLETSKY, D. (1920) On methods of isolation and identification of members of the colon-typhoid group of bacteria. Study of the bacterial action of CR indicator. *J. Bact.* 5 79—87.

GELDREICH, E.E., CLARK, H.F., HUFF, C.B. and BEST, L.C. (1965) Fecal-coliform-organism medium for the membrane filter technique. *J. Am. Wat. Wks. Ass.* **56** 208—214.

PRESSWOOD, W.G. and STRONG, D.K. (1978) Modification of M-FC medium by eliminating rosolic acid. *Appl. Environ. Microbiol.* **36** 90—94.

STEEL, R.G.D. and TORRIE, J.H. (1960) Principles and procedures of statistics with special reference to the biological sciences. McGraw-Hill, New York.