COASTAL POLLUTION MONITORING OF SOUTHERN ITALIAN SITES BY THE MUG ASSAY

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Abstract

A five-year study was carried out on coastal samples collected from Sicilian and Calabrian areas in order to test the performance of an enzyme approach in comparison with the immunofluorescence (IF) and standard culture (FC) methods. The MUG assay was found to be more indicative of the *Escherichia coli* population, as estimated by IF method, than of the whole faecal coliform group determined by plate counts. The usefulness of MUG assay for monitoring mean and heavily polluted areas is suggested.

Keywords: Pollution, bacteria, b-glucuronidase, MUG

Introduction

The detection and enumeration of *Escherichia coli* are of fundamental importance for the control of waterborne diseases and the assessment of the microbiological quality of bathing waters (1). Different reasons support the need for rapid enzymatic methods: real-time assessment would be ideal for the management of water resources and the protection of public health; current standard procedures are labour- and time-consuming and unsuitable for microbiological monitoring; by the incorporation of synthetic enzyme substrates into culture media, rapid monitoring for specific bacteria may be performed, avoiding the need for isolation and confirmation tests (2). The measurement of the β-glucuronidase activity rate by using the 4-methylumbelliferyl-β-D-glucuronide (MUG assay) has been suggested as an indirect alternative approach to estimate the presence of *E. coli* (3). In this paper we report a five-years study carried out on coastal Mediterranean sites, where the reliability of this method for the monitoring of naturally contaminated samples has been tested, in comparison with the direct microscopic count by immunofluorescence (4) and culture methods.

Materials and methods

The MUG assay relies on the determination of β-glucuronidase activity, an enzyme specific of *E. coli* and *Shigella* spp., another Enterobacterium closely related to *E.coli* (2). The fluorogenic compound 4-methylumbelliferyl-β-D-glucuronide (MUG) is the most suitable substrate for the determination of the β–glucuronidase activity; it is enzymatically cleaved into its fluorescent product, 4-methylumbelliferone (MU).

For the enzyme assay, the procedure described by Caruso *et al.* (3) was followed: 10 ml aliquots of a concentrated sample were added at increasing MUG concentrations (from 5 to 50 µM, Sigma) and incubated at 44°C for 3h. Fluorescence intensity measurements were performed at an excitation of 365 nm and 445 nm emission wavelength (3). Data were expressed as the maximum rate of hydrolysis (V_{max}) of MUG (in nmol MU released per 100 ml per h), which provides a measure of the "potential" β-glucuronidase activity present in each sample.

Since 1996, a total of 197 samples were collected from coastal Sicilian and Calabrian sites and analysed by the MUG assay; faecal coliform (FC) and *E.coli* counts were also performed, respectively, by the membrane filtration method on m-FC agar plates and by the microscopic fluorescent antibody (IF) method (4).

Results

The bacterialcounts obtained on samples analysed showed that areas examined differed from each other in their pollution levels; the lowest faecal contamination was measured in Milazzo, followed by Gioia Tauro and Palermo Gulf, where the average values of faecal coliforms and *E.coli* were less than $2.38x10^2$ CFU $100ml^{-1}$ and $2.58x10^4$ cells $100\ ml^{-1}$, respectively. The Straits of Messina suffered a heavier microbial pollution than the other sites; here, FC and *E.coli* reached a maximum of $6.13x10^4$ CFU and $1.87x10^5$ cells $100ml^{-1}$. β-glucuronidase activity values ranged from 1.30 to 5.57 nmol MU/100ml/h.

The statistical analysis of the logarithmic-transformed enzymatic values, plotted versus the immunofluorescence and plate count showed that enzyme values correlated with IF values, and therefore with *E.coli* density, more significantly (R^2=0.1862, n=197, P<0.05) than with FC values (R^2=0.1244, n=197, P<0.05). In order to assay whether the response of the enzymatic assay was affected by the concentration of faecal coliform bacteria, samples were divided into different groups according to their FC concentrations. The regression analysis showed that at high pollution levels, where FC reached values over 10^5 CFU/100ml, the relationship obtained between FC and MUG was more significant (R^2=0.5774, n=79, P<0.05) than that found between FC and IF, while at low pollution levels (FC counts less than 10^3 CFU/100ml), a better relationship between FC and IF was obtained (R^2=0.1401, n=57, P<0.05), as compared to that between FC and MUG (R^2=0.1291, n=57, P<0.05). From 14 to 91% of the variance in the activity rates depended on the variations in the *E.coli* concentration, while a lower percentage (13 to 58%) of the variance in activity rates was explained by the variations in FC counts.

Discussion

Results show that the MUG assay represents a practical approach and can be seen as an alternative to standard culture methods to assess the microbiological quality of seawaters. It may be applied for early warning of faecal pollution episodes, making results available quickly (less than 4 h) and with reduced costs. The highly significant relationship found between log-transformed MUG and IF values suggested thathe enzymatic method is more specific for *E.coli* than for faecal coliforms.A possible explanation is also that the enzyme method is able to detect both viable and viable-but non-culturable (VBNC) cells, still metabolically active, which are estimated by IF only. This may lead to a more accurate estimate of the actual bacterial abundance (5). For heavily polluted samples, the enzymatic values were related more significantly than IF to standard counts, while for less contaminated samples the statistical relationship between IF and FC values was more significant than that found between MUG and FC. We may assume, in fact, that under highly polluted conditions, a high proportion of cells retained their metabolic activity due to the high organic matter availability, while in oligotrophic waters the greater presence of injured/damaged bacteria could account for the poor relationship between MUG and FC (5). Evaluations of metabolic activity may sometimes be different from abundance data, nevertheless the good correlation between the enzyme activity and *E. coli* values supports the determination of β-glucuronidase activity rate as an indirect measure of the presence of *E.coli* and the application of the MUG assay to the detection of this microorganism in marine waters.

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References

1 - Bonadonna L., Chiaretti G., Coccia A.M., and Semproni M., 1997. Valutazione comparativa di procedure analitiche per il rilevamento di *Enterobacteriaceae* in acque marine costiere. *Rapporti ISTISAN* , 97/3: 1-42.

2 - Hartman P.A., 1989. The MUG (Glucuronidase) test for *Escherichia coli* in food and water. Pp. 290-308. *In:* Balows A., Tilton R.C., Turano A. (eds.) Rapid methods and automation in microbiology and immunology. Brixia Academic Press, Brescia.

3 - Caruso G., Crisafi E., and Mancuso M., 2002. Development of an enzyme assay for rapid assessment of *Escherichia coli* in seawaters. *J. Appl. Microbiol.*, 93: 548-556.

4 - Caruso G., Zaccone R., and Crisafi E., 2000. Use of the indirect immunofluorescence technique for detection and enumeration of *Escherichia coli* in seawater samples. *Lett. Appl. Microb.*, 31: 274-278.

5 - George I., Petit M., Theate C., and Servais P., 2001. Use of rapid enzymatic assays to study the distribution of faecal coliforms in the Seine river (France). *Water Sci. Tech.*, 43: 77-80.