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Rapid enzymatic detection of *Escherichia coli* contamination in polluted river water

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Aims: The relationship between the rate of β-D-glucuronidase hydrolysis (GLUase-HR) and the *E. coli* concentration in rivers differing in the extent of faecal pollution was investigated. It was hypothesized that the rate of GLUase-HR is a better surrogate parameter for *E. coli* concentrations than estimated numbers of faecal coliforms (FC).

Methods and Results: The GLUase-HR of the water sample filter residues was determined as the rate of cleavage of 4-methylumbelliferyl-β-D-glucuronide. FC and *E. coli* concentrations were enumerated using mFC and Chromocult Coliform agar, respectively. Regression analysis revealed that a 90% variation of the variable log GLUase-HR was directly related to the variable log *E. coli* concentrations. The observed relationship between the log of the FC count and the log of the GLUase activity could be explained by the hydrolysis activity of the *E. coli* population, as *E. coli* is a part of the FC group.

Conclusions: The data suggest that the log of the GLUase-HR can be used as a surrogate parameter for the log of the *E. coli* concentrations.

Significance and Impact of the Study: GLUase-HR determination may provide a rapid alternative technique to estimate *E. coli* concentrations in freshwaters.

INTRODUCTION

Detailed knowledge of the extent of faecal pollution in aquatic environments is crucial for watershed management activities in order to maintain safe waters for recreational and economic purposes. Techniques which enable rapid and sensitive detection of faecal pollution in environmental freshwaters are thus a prerequisite for efficient water quality monitoring. For this purpose, the determination of *E. coli* contamination in freshwaters can be performed as one of the best means of estimating the degree of recent faecal pollution in temperate regions (Edberg *et al.* 2000).

In recent years a variety of novel techniques have been developed for the detection of *E. coli* in water (Fricker and Fricker 1996). Many are based on the *uidA* gene, or the β-D-glucuronidase (GLUase) enzyme for which it encodes (Martins *et al.* 1993). Although it is possible to target the *uidA* gene directly (Farnleitner *et al.* 2000), usually GLUase activity is used as an enzymatic marker for the identification of *E. coli*. The GLUase activity is detected by its ability to cleave specific chromogenic or fluorogenic artificial substrates added to the culture medium (Manafi 2000) or directly to filtered cells (Van Poucke and Nelis 2000). This approach has led to considerably faster and specific methods for the detection of *E. coli* contamination in freshwaters, although it still requires either significant manipulation and incubation times (Byamukama *et al.* 2000), or sophisticated equipment (Van Poucke and Nelis 2000).

An alternative approach for monitoring faecal pollution is the direct measurement of the rate of the β-D-glucuronidase hydrolysis activity (GLUase-HR) (Fiksdal *et al.* 1994), making use of fluorogenic substrates. The assay can be

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performed within 25 min, thus enabling a rapid detection of faecal pollution (Fiksdal *et al.* 1994). So far, the GLUase-HR has only been correlated to faecal coliform (FC) concentrations and no direct comparisons to *E. coli* concentrations have been carried out (Fiksdal *et al.* 1994; George *et al.* 2000; Farnleitner *et al.* in press). In the present study we investigated the relationship between the GLUase-HR and *E. coli* concentrations in rivers with differing levels of faecal contamination. The study was based on the hypothesis that GLUase-HR might be a better surrogate parameter for *E. coli* concentrations rather than for numbers of faecal coliforms (FC). Consequently, the measurement of GLUase-HR could provide a rapid alternative method to estimate *E. coli* concentrations in freshwater.

MATERIALS AND METHODS

Sampling

Water samples were collected aseptically in 250 ml Pyrex glass bottles (Pyrex, Stone, GB), placed in 4°C cooling boxes and processed within 6 h of collection. Samples were taken from the Austrian rivers Danube, Schwechat, Fischa, and March, and from the Danube Channel of Vienna during 1998 and 1999. Details on the exact sampling schedule and physico-chemical background are given in Farnleitner *et al.* (in press).

Faecal coliforms and determination of *Escherichia coli*

For the determination of FC and *E. coli* concentrations, water samples or dilutions were filtered trough 0·45 μm pore size cellulose nitrate membrane filters (45 mm diameter, Sartorius) (ISO 1990), which were placed on either mFC agar (Biomerieux, Lyon, France) for FC or Chromocult Coliform agar (CCA, Merck, Vienna, Austria) for *E. coli*. The plates were incubated for 24 h in a water bath at 44°C and in a dry heat incubator at 37°C, respectively. All colonies which developed a blue colour on mFC and CCA agar were accounted as FC and *E. coli*, respectively (Byamukama *et al.* 2000; Farnleitner *et al.* 2000).

Measurement of the β-D-glucuronidase hydrolysis rate

A 100 ml sample volume was filtered through 0·45 μm pore size cellulose nitrate membrane filters. For the highly polluted Danube Channel, sample volumes of 20 ml were used. Filters were placed into sterile 250 ml glass bottles (Pyrex) containing 18 ml of phosphate buffer adjusted to pH 6·4 (Fiksdal *et al.* 1994), capped and equilibrated to 37°C. To all prepared flasks, 2 ml of 4-methylumbelliferyl-β-D-glucuronide (MUG, Fluka, Vienna, Austria) substrate solution (1 g MUG l^{-1} supplemented with 1% Triton X-100 v/v) was added. The flasks were tightly closed and incubated at 37°C in a shaking incubator (60 rev min^{-1}). Increases in fluorescence intensity was measured after 10 min, 20 min and 30 min of incubation in a Shimadzu RF 5000 spectrofluorometer, operating at an excitation of 365 nm and an emission of 444 nm, after adding 100 μl 0·1 M NaOH to 2·5 ml aliquots of incubated sample. The relative increase of fluorescence was calculated by least-squares linear regression. To convert the relative fluorescence values to absolute concentrations, methylumbelliferone was added as a standard (MUF, Fluka, Vienna, Austria) with calibration solutions ranging from 10 nM to 800 nM. The GLUase-HR was calculated as MUF production per time (nM MUF min^{-1}). Regression and partial correlation analysis were performed by the statistical software package SPSS Version 7·5.

RESULTS

Regression analysis revealed a highly significant relationship between the log of the GLUase-HR *vs.* the corresponding log of *E. coli* concentrations. A 90% variation of the variable log GLUase-HR was explained by the variable log *E. coli* concentrations (Fig. 1a). Thus the log of the GLUase-HR could be used as an surrogate parameter for the rapid 30 min determination of the log of *E. coli* concentrations in the analysed river waters. Regression analysis of the log of the GLUase-HR *vs.* the log of the FC concentrations also revealed a highly significant relationship (Fig. 1b), but showing a slightly poorer curve fit as compared to the log of the GLUase-HR *vs.* the log of the *E. coli* regression analysis (Fig. 1a). A 87% variation of the variable log GLUase-HR was explained by the variable log FC concentration (Fig. 1b). The observed relationship between the log of the FC and the log of the GLUase activity could be explained by the hydrolysis activity of the *E. coli* population, as *E. coli* is a member of the FC group. This was indicated by a significant decrease of the Pearson's correlation coefficient (r) of 0·93 ($n = 98$; $P < 0·001$) down to an r of 0·28 ($n = 98$; $P = 0·004$) for the log FC *vs.* the log GLUase-HR, when the correlation was controlled for the variations of the log of *E. coli* concentrations by partial correlation.

DISCUSSION

The results of this study show a close relationship between the measured GLUase-HR of the filter residues and the *E. coli* concentrations and indicates that the particle associated GLUase activity of the investigated river waters was mainly due to suspended *E. coli* cells. Previous studies suggested that particle associated GLUase activities in

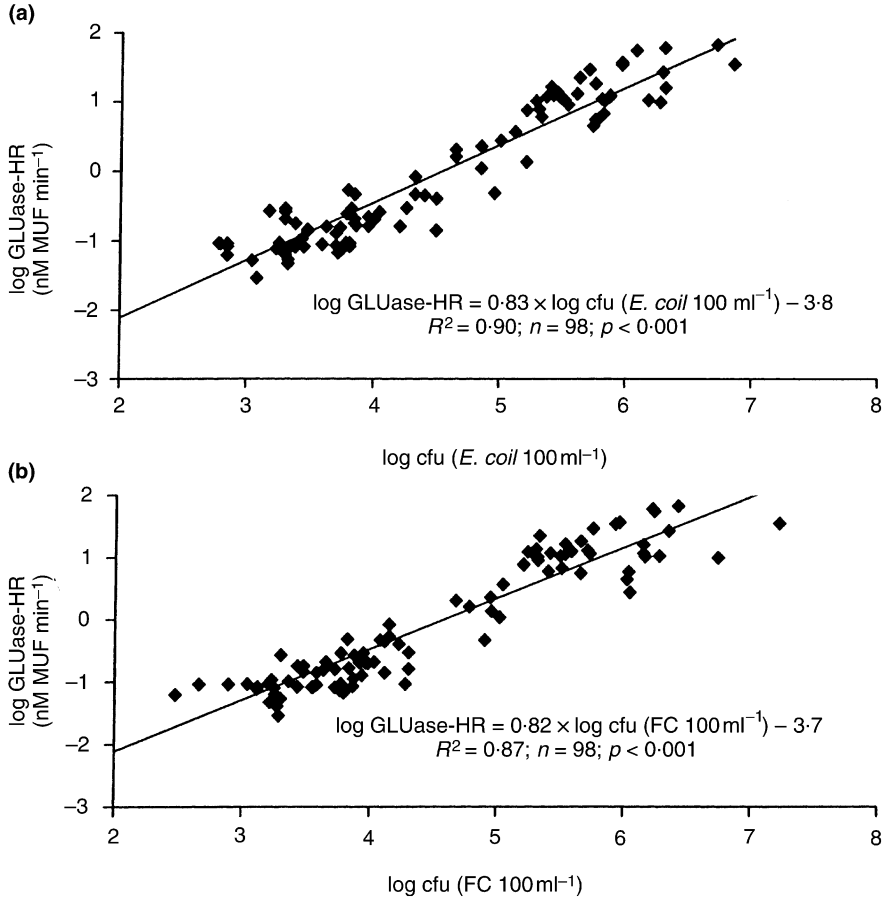

Fig. 1 Regression analysis of the log of the GLUase-HR *vs.* the log of the *E. coli* concentration (a) and the GLUase-HR *vs.* the log of the FC concentration (b)

waters may also be observed from bacteria other than *E. coli* (Tryland and Fiksdal 1998) or from plant material and/or algae (Davies *et al.* 1994). However, potential interfering microbial cells and plant debris must be present in significant abundance in order for it to mask faecal pollution related GLUase activity (Davies *et al.* 1994; Tryland and Fiksdal 1998). This was apparently not the case for the river systems investigated in this study, which is in agreement with previous reports (Fiksdal *et al.* 1994; George *et al.* 2000). It should be mentioned that the dissolved GLUase activity (i.e. the < 0·45 μm diameter fraction) in the investigated river waters accounted for only a relative small amount (median level was approx 10%) in comparison to the corresponding particle associated GLUase activity (Farnleitner *et al.* in press).

It is well known that the FC group mainly consists of *E. coli* and *Klebsiella* sp. (Edberg *et al.* 1997). Furthermore, species of *Klebsiella* do not normally express GLUase activity (Brenner *et al.* 1993). Thus, it is reasonable that the observed GLUase activity of the FC group in this study was mainly due to the presence of *E. coli*. There may be two reasons for the slightly better relationship between GLUase-HR and *E. coli* concentrations (Fig. 1a) as compared to FC concentrations (Fig. 1b). The first is that the inclusion of GLUase negative bacteria (i.e. *Klebsiella* sp.) into the regression analysis, as performed in the case of FC (Fig. 1b), should lead to a poorer relationship between GLUase-HR and *E. coli*. The second is that CC and mFC agar are using differing diagnostic principles for the detection and presumptive identification of the respective bacterial populations. CC agar is principally based on the simultaneous detection of GLUase and β-D-galactosidase (GALase) activity at 37°C dry incubation temperature, whilst mFC agar detects only GALase activity at 44°C wet incubation temperature (ISO 1990; Byamukama *et al.* 2000). Thus, although both diagnostic and selective features used by CC or mFC are generally shared by *E. coli*, methodical differences are likely to influence the results. For example, GLUase negative *E. coli* (Chang *et al.* 1989) are detected by mFC agar but not by GLUase based approaches. Additionally the rather harsh incubation conditions of 44°C, which could lead to an underestimation of injured cells, may affect the relationship between GLUase-HR and the numbers of the *E. coli* FC fraction.

Because this is the first report of the relationship between the log of the GLUase-HR and the log of the *E. coli*

concentrations, comparisons with other studies are only possible for the GLUase-HR *vs.* FC relationships. GLUase-HR regression data from this study revealed a superior goodness of fit ($R^2 = 0{\cdot}87$, Fig. 1b) compared to former studies for natural freshwaters ($R^2 = 0{\cdot}83$) (George *et al.* 2000) and coastal waters ($R^2 = 0{\cdot}77$) (Fiksdal *et al.* 1994), respectively. This may have been caused by the fact that only river and canal waters were investigated in the present study which covered a somewhat smaller range of differing fecal pollution levels as compared to previous studies (Fiksdal *et al.* 1994; George *et al.* 2000). It has been suggested that nonfaecal pollution related GLUase hydrolysis could be significant in stagnant waters like in ponds, whereas flowing waters are less likely to be influenced (Davies *et al.* 1994). In addition, different media, filters and incubation conditions have been used for FC or GLUase-HR determinations in this study compared to the investigations performed previously (Fiksdal *et al.* 1994; George *et al.* 2000).

In both log-log regressions (Fig. 1) a slope smaller than 1 was observed which is in agreement with previous studies (Fiksdal *et al.* 1994; George *et al.* 2000), indicating that the GLUase-HR activity per cultured cell decreased as the number of cultured cells increased for greater polluted environments. A possible explanation for this observation (George *et al.* 2000), is that higher enzymatic activities per culturable cells in less contaminated waters could result in an underestimation of the number of cells when enumerated by plate count due to a higher proportion of active (i.e. cells with detectable GLUase activity) but nonculturable (ABNC) cells in these waters. The higher proportion of ABNC in less polluted waters was suggested being a result of more severe environmental stress factors such as nutrient limitations and enhanced sun light effects due to better light penetration (George *et al.* 2000). In fact, it could be demonstrated with *E. coli* enriched microcosm experiments that determination of GLUase-HR could detect active but nonculturable *E. coli* cells, which had been injured by light stress (George *et al.* 2000).

Our study suggests that the use of the determination of the log of the GLUase-HR has potential as a surrogate parameter for the log of the *E. coli* concentrations. GLUase-HR determination could provide an alternative and rapid method to estimate *E. coli* concentrations in freshwaters. Nevertheless, calibration curves have to be established as an pre requirement for the particular environments to be investigated and the inverse relationship (i.e. *E. coli* as the dependent regression variable of the variable MUGalase) has to be calculated. This technique bears great potential for 'on-line' monitoring of *E. coli* contamination, which has in principle already been demonstrated for other enzymatic activities (Font *et al.* 1997).

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