PROSPECTS FOR MICROBIAL WATER QUALITY MANAGEMENT BASED ON MOLECULAR BIOLOGIC ANALYSIS OF PATHOGENS ON SEDIMENTS AND SUSPENDED SOLIDS.

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ABSTRACT

For practical reasons, the protection of recreational water users against pathogen infection relies traditionally on the enumeration of faecal indicator organisms that insinuate the presence of pathogens. There is considerable uncertainty about the accuracy of such surrogate approaches, and importantly, neither the numbers nor the animal sources of pathogens present can be inferred. Although molecular biologic techniques have been in vogue for some time for the detection of pathogens directly, so far, they have not been adopted for routine environmental water quality management. This communication explores the alternative of detecting pathogens on sediments and suspended solids in the aqueous environment. Microorganisms concentrate on sediment at the interface with the overlying water by a factor of 100 to 1000, and also their survival is prolonged; so the prospects for their identification and enumeration by molecular biologic techniques should be enhanced. The outcome of an experiment to test this proposition is presented, and the benefits for water quality management are discussed.

Keywords: water quality, management, pathogen, sediment, suspended solid, phenotyping, genotyping, PCR

INTRODUCTION

Microbial pollution of the aquatic environment induces an increased public health risk where it is used as a source of potable water, for fish and shellfish farming, and for recreational activities. Such pollution can originate from point sources such as industrial and municipal effluents, or diffuse sources such as land runoff and septic tank seepage that disperse over wide areas (Geldreich 1966). Enteric organisms from grazing animals can enter a stream in runoff from the grazing lands, and animals with access to a stream have been shown to deposit a portion of their daily faecal matter directly therein (Larson et al., 1988). The risk, though, of infection to humans due to animal faecal pollution is perceived generally to be lower than the risk due to human faecal water pollution. Work undertaken recently in the Dargle catchment in Ireland has shown that human activities, especially in urban settlements, contribute a significant microbial load to the river and its tributaries (Bruen et al., 2001); importantly, the results suggested the involvement of human faecal sources.

Sampling the water column alone gives a limited picture of aquatic microbial pollution (American Public Health Association et al., 1998). Microorganisms concentrate on solids either suspended in the water column or in sediments at the interface with the overlying water (Lewis et al., 1986; Pommepuy et al., 1992; Sherer et al., 1988; Tunnicliff and Brickler 1984; Van Donsel and Geldreich 1971). This concentration may proceed by the sorption of the microorganisms to particles such as organic matter (Hendricks 1971) suspended in the water, which may then sediment out. In consequence sediments may sustain faecal indicator organism concentrations orders of magnitude above those in the overlying water (Ashbolt et al., 1993; Van Donsel and Geldreich 1971).

Furthermore, sediment is known to prolong the survival of enteric bacteria (Carrillo et al., 1985; Davies et al., 1995; Hendricks 1971; Hendricks and Morrison 1967; McFeters et al., 1974), perhaps by months (Sherer et al., 1992). This may be because sediment may offer a more favourable chemical and biological environment (Gannon et al., 1983) by protecting bacteria from high salinity (Ghoul et al., 1986), heavy metal toxicity (Jones 1964), UV radiation (Bitton et al., 1972), and attack by bacteriophages (Roper and Marshall 1979). Enteric bacteria have been shown to adapt their genetic expression to stress in seawater (Troussellier et al., 1998). Indeed, several researchers have reported recovering in sediment, enteric organisms that could not be detected in the overlying water (Bitton et al., 1982; Gerba and McLeod, 1976; Gerba et al., 1977; Loutit and Lewis, 1985). Viral survival may not be as strongly promoted by sediment (Ganzer et al., 1998), although virus concentrations in estuarine sediments have been found to correlate with faecal coliform concentrations in the sediments, but not in the overlying water (LaBelle et al., 1980).

Also, sediment-bound bacteria create a potential for elevated bacterial concentrations in the overlying water when re-suspension of sediment occurs (Gary and Adams, 1985; Sherer et al., 1992). Storm water runoff, increased flow velocities, sudden changes in the quality of effluent discharges, and even animal traffic re-suspend bottom sediment in river and estuarine waters. This resuspension is a likely explanation for the erratic variations in faecal indicator organism concentrations frequently observed in water quality monitoring (Sherer et al., 1992; Jawson et al., 1982). Clearly there is merit in paying attention to sediment-associated microorganisms, especially since it may well be that sediment transport mechanisms operate as the principal mechanism of microorganism dispersion in the aquatic environment.

Importantly, the concentrating effect of sediment may enhance the feasibility and ease of the direct determination of sediment-bound pathogens by molecular biologic techniques, i.e. techniques based on the analysis of the nucleic acid content (DNA and RNA) of pathogens (Toze 1999). Also, the possibility of genotyping to identify pathogen sources arises, e.g. human versus farm animal (Bernhard and Field 2000; Dombek et al., 2000; Field et al., 2003; Sinton et al., 1998), although there is debate about this (Malakoff 2002). Underlying these developments is the issue of proper recovery of the nucleic acid content from the aquatic sediment (Hurt et al., 2001; Kreader 1996; Miller 2001; Miller et al., 1999; Moré et al., 1994; Rose et al., 2003); sample work-up is at the heart of this.

METHODS

DNA extraction.

A water sample that included sediment was obtained from the Kilmacanogue River in the Dargle catchment, Co. Wicklow, Ireland using aseptic techniques and agitating the riverbed immediately prior to sampling. After transportation to the laboratory, the sample was re-suspended and filtered through a 0.45 µm sterile 47 mm membrane (Gelman Sciences, Ann Arbor, MI) until the membrane became clogged., A commercial kit (Mo Bio Laboratories Inc. Solano Beach, CA: UltraClean™ Soil DNA Isolation Kit) was used for DNA recovery; 1.0 g of the caked sediment was weighed into a bead tube (emptied of its contents) and centrifuged for 30 seconds at 10,000 g. As much of the supernatant liquid as possible was removed with a pipettor. The bead tube contents were added back and the tube was vortexed gently to mix. Then 60 ml of Solution S1 (re-dissolved if required at 60 °C) were added to the bead tube contents and vortexed briefly, followed by addition of 200 ml of Solution IRS (inhibitor removal solution). The bead tube was secured horizontally to a vortex using masking tape and vortexed at maximum speed for 10 minutes and then centrifuged at 10,000 g for 30 seconds. The supernatant was transferred to a clean micro-centrifuge tube and 250 ml of Solution S2 were added and vortexed for 5 seconds prior to incubating at 4 °C for 5 minutes. Then the micro-centrifuge tube was centrifuged for 1 minute at 10,000 g and the maximum volume of supernatant was transferred to a clean micro-centrifuge tube with care taken not to include any of the pellet. After addition of 1.1 ml of Solution S3 to the supernatant, the tube was vortexed for 5 seconds. Approximately 700 µl of the tube contents were loaded onto a spin filter and centrifuge at 10,000 g for 1 minute. The filtrate was discarded and a further aliquot of supernatant was added to the spin filter and again centrifuged. This was repeated until all the supernatant had been passed through the spin filter. Following addition of 300 µl of Solution S4 the spin filter was centrifuged for 30 seconds at 10,000 g. The filtrate was discarded and the spin filter was centrifuged again for 1 minute. The spin filter was placed carefully in a clean micro-centrifuge tube and 50 ml of Solution S5 were added to the centre of the white filter membrane. After centrifugation for 30 seconds the spin filter was discarded, and 1.0 µl of the filtrate was used for a 50 µl polymerase chain reaction (PCR).

PCR conditions.

Reaction mixtures for PCR contained 1 x PCR buffer, each deoxynucleoside triphosphate at a concentration of 200 µM, 1.25 mM $MgCl_2$, each primer at a concentration of 0.06 µM, 1.52 µM BSA, and 2.5 U of Taq DNA polymerase (Promega, Madison, WI, USA) in a final volume of 50 µl. DNA amplification was performed with a thermocycler (Techne PHC-3, Cambridge, UK) by using the following program: 5 minutes at 94 ºC, followed by 30 cycles consisting of denaturation (1 minute at 94 ºC), annealing (1 minute at 52 ºC), and extension (1.5 minutes at 72 ºC) and a final extension at 72 ºC for 5 minutes.

Gel electrophoresis:

Aliquots of PCR mixtures (10 µl) were electrophoresed in 2% agarose in 1 x TAE buffer with ethidium bromide to verify DNA amplification.

Gel documentation:

A UV transilluminator (UVP Life Sciences, Cambridge, UK) was used to visualize the DNA electrophoretogram and to record a gel image.

RESULTS AND DISCUSSION

The products of the PCR amplification of DNA recovered from the river sample using *E. coli* primers for a conserved 16S rDNA region, an *E. coli* 16S rDNA control sample and a 100 base-pair ladder sample were separated by electrophoresis on a 2% agarose gel with ethidium bromide incorporated in the gel for DNA visualisation. An inverted image of the resulting gel obtained under UV light is shown in Figure 1.

The DNA fragments of the 100 base-pair ladder were separated effectively (lane 1), indicating that the electrophoretic separation had been successful. A single 1500 base pair band is visible in lane 2 as expected for the positive control PCR product, evidencing the success of the PCR. Two bands are detected in lane 3; one of these is aligned with the 1500 base pair positive control. The presence of the faint, lower-size band (about 650 base pair size) was unexpected, and is currently unexplained. However it is clear that the recovery and amplification of *E. coli* DNA from the river sediment sample was successful.

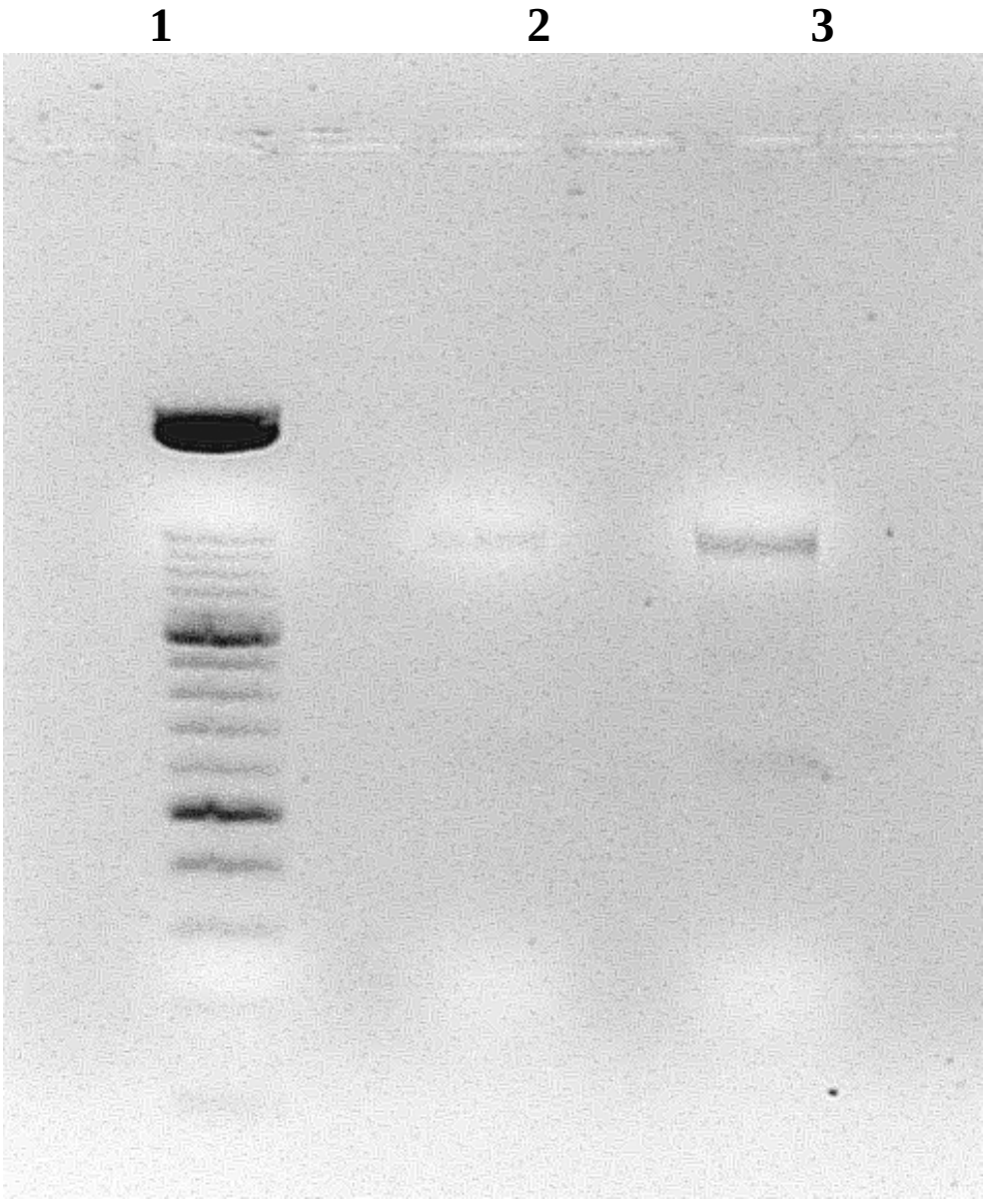

Figure 1. Electrophoretogram of 16S rDNA after amplification of DNA recovered from a river sediment sample by polymerase chain reaction.

Lane 1: 1.5 µl of 100 base-pair ladder sample.
Lane 2: 10 µl of positive control reaction.
Lane 3: 10 µl of river-sample reaction.

CONCLUSIONS

Molecular biologic techniques have been in vogue for some time for the detection of pathogens directly in water samples (Bej and Toranzos, 1997; Gerba et al., 1988; Margolin et al., 1985; Steffan et al., 1989); to achieve detection water samples must first be concentrated; this has been achieved, for example, by filtration with a positively-charged nylon membrane (Gilgen et al. 1997), or by adsorption/elution using silica particles (Pallin et al. 1997). Sediments or suspended solids can be isolated with convenience from relatively small volumes by standard filtration or centrifugation methods, and also stored readily. As outlined above, the challenge is in the recovery and amplification of sediment nucleic acid; success in isolating and in amplifying 16S rDNA from a river sediment sample is reported here. Present work is aimed at exploring the use of genotyping based on sediment and suspended solids, with a view to understanding more fully the sources of diffuse microbial pollution in the Dargle catchment.

While there is still much to be done to establish this technology in water quality management, it is worth pointing out that molecular biologic techniques are routinely used in clinical and in food-safety laboratories, and as is well known, in forensic laboratories. It can be expected that the management information to be obtained will be of similar character to that obtained in these other areas, and lead to more definitive water quality management.

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