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Evaluation of an anion exchange resin-based method for concentration of F-RNA coliphages (enteric virus indicators) from water samples

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A B S T R A C T

Enteric viral contaminants in water represent a public health concern, thus methods for detecting these viruses or their indicator microorganisms are needed. Because enteric viruses and their viral indicators are often found at low concentrations in water, their detection requires upfront concentration methods. In this study, a strong basic anion exchange resin was evaluated as an adsorbent material for the concentration of F-RNA coliphages (MS2, Qβ, GA, and HB-P22). These coliphages are recognized as enteric virus surrogates and fecal indicator organisms. Following adsorption of the coliphages from 50 ml water samples, direct RNA isolation and real time RT-PCR detection were performed. In water samples containing 10^5 pfu/ml of the F-RNA coliphages, the anion exchange resin (IRA-900) adsorbed over 96.7% of the coliphages present, improving real time RT-PCR detection by 5–7 cycles compared to direct testing. F-RNA coliphage RNA recovery using the integrated method ranged from 12.6% to 77.1%. Resin-based concentration of samples with low levels of the F-RNA coliphages allowed for 10^0 pfu/ml (MS2 and Qβ) and 10^{-1} pfu/ml (GA and HB-P22) to be detected. The resin-based method offers considerable advantages in cost, speed, simplicity and field adaptability.

1. Introduction

There are more than 140 enteric viruses excreted in human and animal wastes (Leclerc et al., 2000; Fong and Lipp, 2005), and water is recognized as one of the most important vehicles for their transmission (Kelly, 1953). Individuals may be exposed to such contamination through drinking water, recreational water, irrigation and food processing water, or seafood produced in impacted environments (Bosch et al., 2008). Thus, efficacious strategies for enteric virus detection in water are needed to assess potential health hazards, assist epidemiological investigations, understand the ecology and transmission of different viruses and to measure the effectiveness of water treatments (Wyn-Jones and Sellwood, 2001; Bosch et al., 2008). Ideally, such a method would be sensitive, rapid, reliable, inexpensive, compatible with different water types and technically feasible for a large number of samples.

Given the variety of viral enteric pathogens and their particular growth requirements, detection of all possible enteric viruses in a water sample is technically challenging, time consuming and expensive (Leclerc et al., 2000). An alternative to determine the risk of enteric virus contamination of water is to detect viral indicators of fecal contamination, such as F-RNA coliphages. These coliphages are members of the family *Leviviridae*, have single stranded positive sense RNA genomes and are non-pathogenic for humans (Jofre et al., 2011). In nature, F-RNA coliphages are shed exclusively in feces (Havelaar et al., 1993; Grabow, 2001), share structural features with enteric viruses and display similar chemical/environmental sensitivities. These factors make F-RNA coliphages exceptional indicators of fecal contamination as well as enteric virus model organisms (Zerda et al., 1985; Lukasik et al., 2000; Charles et al., 2009; Ikner et al., 2011; Jofre et al., 2011). For example, F-RNA coliphages are useful indicators of norovirus and other enteric viruses in molluscan shellfish and fresh water (Havelaar et al., 1993; Doré et al., 2000). In addition, the F-RNA coliphage MS2 was used as a surrogate for human enteric viruses in studies of wastewater disinfection and viral persistence in surface water and ground water (Tree et al., 2005; Bae and Schwab, 2008).

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Enteric viruses and their viral indicators are often present in naturally contaminated water at such low concentrations that they are undetectable without upfront concentration, even when modern molecular methods such as quantitative real time PCR are utilized. Several methods exist to concentrate viruses from water, which exploit morphological features of the virion(s). These features include viral colloidal nature, particle sizes and the chemical and physical properties of the virion surface, most notably electrostatic charge (Wyn-Jones and Sellwood, 2001; Ikner and Gerba, 2012). Electronegative and electropositive filters (in membrane or cartridge formats) are currently the most commonly used viral concentrators for water analysis (Ikner and Gerba, 2012). Once viruses are adsorbed on the filter surface, concentration is accomplished by eluting adsorbed viruses. The elution process, which is often inefficient, utilizes extreme pH or high protein content solutions, which have been reported to inactivate the target virus and/or inhibit downstream detection (Grabow, 2001; Ikner and Gerba, 2012). Additionally, 10–300 ml of buffer are required for elution of the filters, and as molecular detection techniques are intended to analyze very small volumes of sample (5–10 μl per reaction), this process reduces the sensitivity of downstream molecular detection and necessitates a secondary concentration step, increasing processing time and cost (Wyn-Jones and Sellwood, 2001; Wu et al., 2011; Ikner and Gerba, 2012).

Alternative materials have been used for virus adsorption including ion exchange resins (Muller, 1950), gauze pads (Kelly, 1953), polyelectrolites (Wallis et al., 1971), glass powder (Schwartzbrod and Lucena-Gutierrez, 1978) and fiber glass (Joret et al., 1980). Nonetheless, when these materials were tested, molecular techniques were not developed, and their applicability was limited to culture-based techniques for virus detection.

Virus concentration methods should be applicable to a large range of viruses, different water matrices, have a high recovery rate, be easy to perform, cost effective and rapid. Additionally, such methods should provide a small volume of concentrate free of inhibitors that can complicate downstream detection (Grabow, 2001; Wyn-Jones and Sellwood, 2001;Bosch et al., 2008). In this study, we propose a method to concentrate viruses from water using an anion exchange resin (IRA-900) dispersed in the sample to facilitate the adsorption of negatively charged viruses. Isolation of viral nucleic acids can be performed directly from the resin, without the need for large-volume elutions, with the resulting sample compatible with real time RT-PCR detection. To test this methodology, four different F-RNA coliphages (each belonging to one of the four F-RNA genogroups) were used, providing examples of viruses with similar structures to enteric viruses and having diverse isoelectric points (pI) ranging from 2.1 to 5.3 (Michen and Graule, 2010). This system proved to be effective for F-RNA coliphage adsorption and detection from 50 ml water samples, providing an alternative to filter-based concentration methods.

2. Materials and methods

2.1. Coliphages and bacterial strains

F-RNA coliphages from genogroup I (MS2 ATCC 15597-B1), genogroup III (Qβ ATCC 23631-B1) and the bacterial host *E. coli* HS(pFamp)R (ATCC 700891) were obtained from the American Type Culture Collection (Manassas, VA, USA). F-RNA coliphage GA (genogroup II) and HB-P22 (genogroup IV) were kindly provided by Stephanie Friedman (U. S. EPA, Gulf Breeze, FL, USA). Coliphages were propagated by infection of a logarithmic phase *E. coli* culture in tryptic soy broth supplemented with 5 mM magnesium chloride and 50 μg/ml of ampicillin and streptomycin (Sigma–Aldrich, Saint Louis, MO, USA), at a multiplicity of infection of 5–10. After 18–24 h of incubation with shaking at 37 °C, coliphage stocks were prepared by adding 10% v/v chloroform to the infected bacterial culture, followed by centrifugation at 6000 × *g* for 25 min at 4 °C to remove cellular debris. The supernatants were filtered through a 0.22 μm low protein-binding filter (PALL Life Sciences, Ann Arbor, MI, USA) and stored at 4 °C. Coliphage stocks were regularly enumerated using the double agar overlay plaque assay (Hershey et al., 1943) as modified by Kropinski et al. (2008).

2.2. Adsorption of F-RNA coliphages to anion exchange resin

Tap water from the Fort Collins, CO municipal water supply was dechlorinated using 50 mg/l of sodium thiosulphate pentahydrate (Mallinckrodt Baker Phillipsburg, NJ, USA). Serial ten-fold dilutions of coliphage stocks were prepared in lambda buffer [0.58% NaCl, 0.2% $MgSO_4$ heptahydrate, 0.01% gelatin, and 0.05 M Tris–HCl, pH 7.5 (Sigma–Aldrich)] and 1 ml of the appropriate dilution was used to inoculate 50 ml water samples (final coliphage concentrations ranging from 10^{-1} to 10^5 pfu/ml).

To determine the adsorption efficiency of the anion-exchange resin, experiments to measure the reduction of phage concentration in the water sample due to resin retention were performed as follows: 0.5 g of Amberlite IRA-900 anion exchange resin (Polysciences, Warrington, PA, USA) was added to 50 ml water samples ($n = 3$) spiked with a final concentration of 10^5 pfu/ml of each F-RNA coliphage. Samples were incubated for 90 min at room temperature in polypropylene conical tubes with gentle mixing (36 rpm) using a rotating sample mixer (Dynal Biotech, Lake Success, NY, USA). Water samples for RNA isolation (140 μl) and for plaque assay (100 μl) were taken at 0, 30, 60 and 90 min. At the end of the incubation period (90 min), resin was collected for direct RNA isolation of the adsorbed coliphages. To assess coliphage stability during the process, another 50 ml sample of water with the same coliphage concentration was incubated under the same conditions but with no resin and processed as above. All experiments were done in three replicates.

To measure the sensitivity of the resin-based concentration method, 50 ml water samples inoculated with different concentrations of coliphages, ranging from 10^{-1} to 10^2 pfu/ml ($n = 3$ for each coliphage at the specified concentrations) were incubated with 0.5 g resin as described above. RNA isolation was performed on water samples at 0 min and 90 min.

2.3. RNA isolation

For isolation of coliphage RNA from water samples, 140 μl of each sample was processed using the QIAmp viral RNA kit© (Qiagen, Valencia, CA, USA) according to the manufacturer's instructions. For direct RNA isolation of the coliphages adsorbed onto the resin, the resin was allowed to settle for one min, and water was decanted. Excess liquid was removed using a pipette tip, and 560 μl of AVL buffer (from the QIAmp kit) was added to the resin. After a 10 min incubation step with occasional agitation, the supernatants were transferred to 1.5 ml Eppendorf tubes and RNA isolation was performed according to manufacturer's instructions. For both water and resin samples, the RNA was eluted in 60 μl of AVE buffer (from the QIAmp kit).

2.4. Nucleic acid detection

Real time reverse transcription PCR (RT-PCR) to detect the four different genogroups of coliphages were performed in a Step One Plus thermocycler (Applied Biosystems, Foster City, CA, USA) using the One Step RT-PCR kit (Qiagen). For MS2, GA and HB-P22 (coliphage genogroups I, II and IV, respectively), detection was achieved as described previously (Pérez-Méndez et al., 2013) with