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Concentration of enteric virus indicator from seawater using granular activated carbon

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A B S T R A C T

Fecal contamination of shellfish growing seawater with enteric viruses is often associated with human outbreaks of gastroenteritis. Male specific bacteriophage MS2 is correlated with those of enteric viruses in a wide range of water environments and has been used widely as a surrogate for pathogenic waterborne viruses. Since viruses in contaminated water are usually at low levels, the development of methods to concentrate viruses from water is crucial for detection purposes. In the present study, granular activated carbon was evaluated for concentration of MS2 from artificial seawater, and different parameters of the seawater were also compared. Recovery of MS2 from warm seawater (37 °C) was found to be significantly greater than from cold seawater (4 and 20 °C), and even greater than from fresh water (4, 20 and 37 °C); the difference between seawater and fresh water became less profound when the temperatures of both were below 37 °C. Although not of statistical significance, recovery of MS2 from low salinity seawater (10 and 20 parts per thousand, ppt) was greater than from high salinity seawater (30 and 40 ppt). One gram of granular activated carbon was able to extract 6-log plaque forming units (PFU) of MS2 from 500 ml seawater at 37 °C. This study demonstrated that granular activated carbon can concentrate an enteric virus indicator from shellfish growing seawater effectively.

1. Introduction

Enteric viruses are responsible for a large proportion of food and water-borne illnesses. These viruses are transmitted to humans via the fecal–oral route, usually from contaminated water or foods such as raw shellfish (Bosch, 1998; Richards, 2001; Dubois et al., 2002; Kingsley et al., 2002). Viral contaminated shellfish can be a public health concern, as outbreaks of gastroenteritis have occurred among consumers of shellfish harvested from fecal polluted waters. Shellfish are filter feeders that can actively bio-concentrate human enteric viral pathogens from contaminated growing waters by as much as 100 fold and retain the infectious virus particles for up to one month (Nappier et al., 2008; Wang et al., 2008; McLeod et al., 2009; Le Guyader et al., 2012). Even point source discharge of human waste from commercial and recreational vessels can result in viral contamination of approved shellfish beds without observation of increase in fecal coliform in marine water samples. The National Shellfish Sanitation Program recommends immediate closure of shellfish growing area when enteric viral pathogens are suspected (National Shellfish Sanitation Program, 2011).

A rapid detection of viral contamination in water environments can prevent large scale economic loss due to closure of recreation beach and shellfish harvesting sites, and can identify the source of contamination within a short time for public safety. Traditional indicator organisms like fecal coliform behave as good indicators for enteric bacteria but poor indicators for enteric viruses. In contrast, male-specific bacteriophages have attracted interest as useful alternatives to bacterial indicators because of their morphology and survival characteristic resemblance to enteric viruses. Several studies have confirmed that, for monitoring purposes, phages are reliable indicators of human enteric viruses in seafood and seafood harvesting waters (Meschke and Sobsey, 2003; Blaise-Boisseau et al., 2010; DePaola et al., 2010; Love et al., 2010; Serracca et al., 2010). As a group I male-specific RNA coliphage that infects *Escherichia coli* (Nappier et al., 2008), Enterobacteria phage MS2 is used widely as a surrogate for enteric viruses, and it is proved to offer a reliable way to monitor false-negative results. Its survival and inactivation rates are similar to common enteric viruses (Allwood et al., 2003; Huertas et al., 2003; Shin and Sobsey, 2003; Tree et al., 2003; Bae and Schwab, 2006; Katz and Margolin, 2007). MS2 is a member of the family *Leviviridae* and genus *Levivirus* and has a small, icosahedral capsid of 26.0–26.6 nm in diameter (Van Duin, 1988). It infects *E. coli* by attaching to the sex pili of *E. coli* and multiplies inside the bacteria by making use of some or all of the host biosynthetic machinery. Its 3.5 kb single stranded RNA genome

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acts as messenger RNA and is responsible for encoding lysis protein that lyse the host bacteria upon completion of assembly (Strauss and Sinsheimer, 1963; Bae and Schwab, 2008; Carter and Saunders, 2007). Unlike most human pathogenic enteric viruses, which cannot be cultured, male-specific RNA coliphage can be detected by culture assays and molecular biology assays.

Numbers of viruses and phages in contaminated food or water are usually too low for detection; however, even one infectious virus may cause illness (Schiff et al., 1984; Fong and Lipp, 2005). Therefore, large volumes of water (10–20 L) needs to be concentrated before analysis can be carried out. Different types of filters and filtration methods have been used to collect and concentrate viral particles from water samples (Pallin et al., 1997; Gantzer et al., 1999; Lipp et al., 2001; Griffin et al., 2003; Fong and Lipp, 2005). Because of the small size of viral particles, mechanical filtration is often not possible; therefore, adsorption–elution methods are employed. These involve manipulation of charges on the virus surface, using pH changes to maximize their adsorption to charged filters (Pallin et al., 1997; Lipp et al., 2001; Fong and Lipp, 2005). Adsorption–elution of viruses with an electropositive filter is one of the most commonly used techniques (American Public Health Association, 1995; Katayama et al., 2002; Fong and Lipp, 2005). These filters require no manipulation of pH because most enteric viruses are negatively charged at ambient pH (Lipp et al., 2001). However, electropositive filters are easily clogged and have low recovery rates for viruses in marine water; the presence of salt and alkalinity of seawater cause low absorption of viruses to the filters (Lukasik et al., 2000). Under ambient conditions, most of the enteric viruses are negatively charged and are known to adsorb to an electronegative filter in the presence of Mg_2^+ or other multivalent cations, or under acid conditions, while the recovery of viruses is not always easy (Sobsey et al., 1973; Sobsey et al., 1995; Fong and Lipp, 2005). Ultrafiltration methods on the other hand require minimal manipulation of water; but are less cost- and time-effective than adsorption-elution because of the high cost of equipment and limitations on the volume of sample that can be concentrated at one time (Jiang et al., 2001; Fong and Lipp, 2005). Concentrated or eluted water samples usually are further concentrated and purified to reduce the final volume of samples to 1 or 2 ml for processing (Jiang et al., 2001; Lipp et al., 2001; Noble and Fuhrman, 2001; Katayama et al., 2002; Haramoto et al., 2004; Fong and Lipp, 2005). Procedures of secondary concentration have often been reported to cause inhibitory effects in PCR (Arnal et al., 1999; Fong and Lipp, 2005).

In an attempt to seek an alternate method that would reduce the time and cost of virus concentration and avoid blockage issues, the use of granular activated carbon was investigated. Activated carbon consists of a wide range of amorphous carbonaceous materials with a high degree of porosity and extended inter-particulate area obtained by combustion, partial combustion, or thermal decomposition (Bansal and Meenakshi, 2010). Activated carbon exists in granular or powder forms. The granular from has a large internal surface area and small pores while the finely divided powder form is associated with larger pore diameters and smaller internal surface area. Granular activated carbon is used commonly in water reclamation for organic matter removal, and the pore-size distribution of granular activated carbon was revealed to be the key contributor to the adsorption capacity and rate of uptake of contaminants (Lee et al., 1981). Various studies suggested the potential of activated carbon on virus adsorption from waste water (Cookson and Wheeler, 1967; Gerba et al., 1975; Powell et al., 2000). Activated carbon was documented to remove 99% of viruses from the water, and adsorb up to 12 logs of viruses per gram, while the adsorbed virus maintains infectivity (Cookson and Wheeler, 1967; Powell et al., 2000). The adsorption capacity and lack of virucidle activity of activated carbon rendered it suitable in concentration of enteric viruses from seawater. As no previous studies have conducted their experiment in seawater, this report compares fresh water with seawater, and the impact of different parameters of the seawater (temperature, salinity and pH) on granular activated carbon.

2. Materials and methods

2.1. Bacteriophages and host cells

E. coli (ATCC 15597) was grown at 37 °C for 6 h. The growth media of *E. coli* contained 10 g tryptone, 1 g yeast extract, 8 g NaCl per liter of medium; after the medium was cooled to 50 °C, the following supplements glucose (0.1%), $CaCl_2$ (2 mM) and thiamine (10 μg/ml) were added to the cooled medium. Bacteriophage MS2 (ATCC 15597-B1) was propagated in the exponentially growing *E. coli* culture for 16 h at 37 °C. The overnight culture of MS2 was clarified by centrifugation at 2300 × *g* for 10 min and filtration through a 0.45 μm filter. Serial dilutions of 10^{-1} to 10^{-9} were made.

2.2. Viral titration

The titer of the MS2 culture was determined by plaque assay. Briefly, 10 ml of bottom agar (1%) was poured in 100 × 15 mm petri dishes and 10 ml agar (0.45%) containing *E. coli* 6 h culture was poured on top. Supplements of glucose, $CaCl_2$ and thiamine were added to both layers at final concentrations of 0.1%, 2 mM and 10 μg/ml. MS2 dilutions (30 μl) were then spread on top of the agar with a hockey stick. All plates were incubated at 37 °C overnight before examined for plaques.

2.3. Quantitative PCR

PCR was performed in a total volume of 25 μl with a SmartCycler (Cepheid, Sunnyvale, CA, USA). In each reaction, 10 μl of RNA was added to 15 μl of mixture containing 0.2 μl 125× RT Enzyme Mix (ArrayScriptTM UP Reverse Transcriptase, RNase inhibitor), 12.5 μl 2× RT-PCR Mix (SYBR® Green I dye, AmpliTaq Gold® DNA Polymerase, Ultra-pure, dNTPs, ROXTM passive reference, Optimized buffer components), 0.2 mM of each primer, 1.5 μl RNase-free water. The thermocycling condition and primer sequences were based on method by O'Connell et al. (2006). PCR thermocycling condition was as follows: 48 °C for 30 min, 95 °C for 10 min, 40 cycles of (95 °C for 15 s, 60 °C for 1 min), then 95 °C for 1 min, and 65 °C for 2 min. The primers were as follows: forward: 5′ GTC GCG GTA ATT GGC GC 3′; reverse: 5′ GGC CAC GTG TTT TGA TCG A 3′.

2.4. Standard curve

Standard curve was performed by testing 10-fold dilutions of viral solution of MS2. The RNA was extracted from 10^0 to 10^{-9} MS2 dilutions using QIAamp Viral RNA Mini Kit (Qiagen Inc., Valencia, CA, USA). The PCR standard curve was constructed from quantifying all the RNA samples in one reaction and the plaque forming unit of each sample was correlated to the cycle threshold (Ct) value. The Ct values of each dilution amplified by PCR were plotted as a function of the logarithm of the starting quantity of phages in PFU (Jothikumar et al., 1998; Shin and Sobsey, 2003; Ogorzaly and Gantzer, 2006; Langleta et al., 2009; Bae and Schwab, 2008; Blaise-Boisseau et al., 2010; Furiga et al., 2011; Liu et al., 2011). The quantity of phages can also be expressed in genome copies (Dreier et al., 2005; Rolfe et al., 2007; Pecson et al., 2009; Dong et al., 2010). Rolfe et al. (2007) calculated the genome copy number of MS2 to be 9.18 per PFU.