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Evaluation of viral concentration methods from irrigation and processing water

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Four viral concentration methods were evaluated for their efficiency in recovering murine norovirus-1 (MNV-1) (surrogate for human noroviruses (NoV)) and MS2 bacteriophages from processing water (1 L) and four different types of irrigation water (bore hole water, rain water, open well and river water) (2–5 L). Three methods were based on the viral adsorption and elution principle, two methods using an electronegative HA-membrane (Katayama et al., 2002), one method using an electropositive Zetapor membrane according to CEN/TC275/WG6/TAG4 and the fourth method was based on size exclusion using a tangential flow filtration system. Detection of MNV-1 was achieved by real-time RT-PCR and detection of MS2 by double-layer plaque assay.

For the recovery of MNV-1, the method using an electronegative HA-filter in combination with an elution buffer earlier optimized by Hamza et al. (2009) (Method 1) performed best for all types of water (recovery: 5.8–21.9%). In case of MS2 detection, the best method depended upon the type of water although Method 1 provided the most consistent recovery.

To complete this evaluation, the Method 1 was evaluated further for the concentration of human enteric viruses (GI and GII NoV, hepatitis A virus (HAV) and rotaviruses) in the same five types of water. Although detection of rotaviruses (RV) was somewhat less efficient, Method 1 proved reliable for the detection of NoV and HAV in all water types. Mean recovery efficiencies ranging from 4.8% for detection of GI NoV in open well water to 32.1% for detection of HAV in bore hole water, depending on the water type and the viral pathogen analyzed.

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1. Introduction

Viral pathogens, such as human infectious NoV and HAV, play a significant role in food borne outbreaks throughout Europe and the U.S. (CDC, 2009; EFSA, 2010). Fresh produce are, next to shellfish and ready-to-eat foods, recognized as an important vehicle in the transmission of food borne viral outbreaks (FAO/WHO, 2008).

Fresh produce can be contaminated at the pre-harvest stage by contact with viral contaminated water or sludge or both at the pre-harvest and post-harvest stage by contact with asymptomatic or symptomatic infected food handlers, contaminated processing water or surfaces (Baert et al., 2009; Carter, 2005; Leon-Felix et al., 2010; Seymour and Appleton, 2001). Whereas human activity and thus food handler's contamination is an established source of food borne viruses and can be controlled by good hygienic practices and training, little knowledge is available on the prevalence of food borne viruses in irrigation water or water used in post-harvest processes such as washing and rinsing, and thus its role for acting as a vehicle of transmission to fresh produce crops. Several sources of water are applied for irrigation of crops and this may range from ground water and collected rain fall (general assumed to be of good and even potable water quality) to surface water (streams, rivers) and may include in some regions also insufficiently treated wastewater with variable microbial quality (Pachepsky et al., 2011). Microbial quality is generally measured by the use of bacterial indicator organisms such as coliforms, fecal coliforms and *Escherichia coli* but these indicator organisms may not be an accurate reflection of enteric virus presence (Jurzik et al., 2010; Steele and Odumeru, 2004). The results of previous screenings in water have shown that human enteric viruses are abundantly present in diverse ranges of water sources in the environment worldwide (Lodder et al., 2010; Miagostovich et al., 2008; Victoria et al., 2010; Wyn-Jones et al., 2011). River water samples are likely to be contaminated as they are fed continuously with effluents of wastewater treatment plants, which are optimized for the removal of bacteria and are less effective in removing viruses (da Silva et al., 2007; Hewitt et al., 2011; Maunula et al., 2012; Ueki et al., 2005). Even though river water is one of the irrigation water sources that is most likely to be contaminated with hazardous microorganisms, this water type is in most parts of the world most commonly used for irrigation of salads which are consumed raw (Knox et al., 2011). Even in water sources considered relatively safe for bacterial contamination, such as ground water, viruses could be detected (Cheong et al., 2009; Park et al., 2010; Steyer et al., 2011). At present

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few studies have looked into the prevalence of food borne viruses such as NoV, HAV and RV in sources of irrigation water in order to estimate the possibility of viral contamination of fresh produce irrigated with these waters, although a link has been made between the detection of human enteric viruses on the irrigated vegetables and in the irrigation water (Cheong et al., 2009; van Zyl et al., 2006). This in part by the need of laborious viral concentration and detection methods and the insufficient knowledge on their performance in these types of water matrices. As such, most studies that concentrate on the performance of different viral concentration methods from water, focus on other types of water such as standardized water (e.g. distilled water) (Lee et al., 2011), drinking water, source water for drinking water production (Gibson and Schwab, 2011), influent and effluent waters in order to evaluate the efficiency of wastewater treatment plants in their removal of viruses (Albinana-Gimenez et al., 2009; Wyn-Jones et al., 2000), and seawater as production area of bivalve mollusks or recreational zone (Gibbons et al., 2010).

The aim of this study was to select an appropriate viral concentration method for monitoring the presence of food borne viruses in different types of irrigation water and also post-harvest washing water used in fresh cut produce industry.

To our knowledge this is the first study that attempts to evaluate different viral concentration methods for the detection of food borne viruses in various types of water applied commonly in the horticultural sector during agricultural production and further processing.

In the current study two different approaches to concentrate viruses in water were evaluated. Three methods applying the VIrus ADsorption and ELution (VIRADEL) principle, based on a protocol described by CEN/TC275/WG6/TAG4, Hamza et al. (2009), Katayama et al. (2002) and Wyn-Jones et al. (2011), were compared to a single method based on the principle of size-exclusion, more specifically ultra-filtration using a tangential flow filtration system previously validated by Ceeram (La Chapelle-sur-Erdre, France).

MNV-1 and bacteriophage MS2 served as human enteric viral pathogen surrogates for the evaluation of the four viral concentration methods, in four types of irrigation water (bore hole water, rain water, open well water, river water) and one type of processing water. A wide range of irrigation water types were chosen as it is clear from previous experiments that a single viral concentration method can show different recovery efficiencies depending on the type of water examined (Haramoto et al., 2009; Lewis et al., 2000; Victoria et al., 2009). For the detection of MNV-1, molecular methods were chosen for detection since for the detection of major food/water borne enteric viruses an appropriate cell culture does not exist (Koopmans and Duizer, 2004). Based on the comparison of the different viral concentration methods, one method was selected for further evaluation with a broad panel of human enteric viruses, including GI and GII NoV, RV and HAV, in the same five types of water.

2. Materials and methods

2.1. Water samples

Four primary concentration methods were tested for their efficiency in recovering viruses from various types of water.

For the purpose of this study all types of irrigation water samples – bore hole water, rain water, open well water and river water – were collected in an agricultural area surrounding the city of Ghent (Belgium). Bore hole water was taken from a 9 m deep well in sandy soil. Rain water was collected from an underground tank where the water was stored after it was collected initially from a roof top situated in an agricultural area. Open well water was collected from an open well situated in a pasture. River water was taken from the 'Oude Leie' (an old cut off part of the river 'Leie') before entering Ghent, while processing water was taken from a local fresh cut lettuce processing plant from the water bath at the end of the washing stage of mixed salad. The processing water was dechlorinated by the addition of 100 mg/L sodium thiosulfate (Sigma–Aldrich, Steinheim, Germany). Water samples of each source were taken on two different occasions: during spring and at the end of the summer. Sampling volumes of 1–5 L were processed when possible (depending on the method and type of water sample).

Water quality parameters analyzed just before filtration were pH and total suspended solids (TSS) according to Standard Methods (1998) (APHA, 1998). Samples were stored at 4 °C for a maximum of 48 h before use in the experimental set-up.

2.2. Artificial contamination of water samples

For the initial comparison of the four different viral concentration methods, water samples were contaminated artificially with MNV-1, a human NoV surrogate kindly provided by Prof. H.W. Virgin, and with MS2 bacteriophages, kindly provided by the Flemish Institute of Biotechnology (VIB, Ghent, Belgium). MNV-1 was cultured as described earlier (Wobus et al., 2004) and MS2 was cultured according to ISO 10705-1 (ISO, 1995). Stock dilutions of MNV-1 and MS2 were prepared in respectively PBS (Lonza, Verviers, Belgium) and PPS and stored in aliquots at −80 °C until use. Concentrations of MNV-1 genomic copies and MS2 titer (in PFU) were respectively determined by real-time RT-PCR (Baert et al., 2008b; Stals et al., 2009) and double-layer plaque assay, ISO 10705-1 (ISO, 1995).

Water samples were spiked to a final concentration of approx. 7 log MNV-1 genomic copies/L and approx. 7 log MS2 PFU/L.

For the further evaluation of the selected viral concentration method, the five different types of water were contaminated artificially with NoV, RV and HAV. NoV GI.4 and GII.4 and rotavirus G1P[8] stool samples were kindly provided by the National Institute for Public Health and the Environment (RIVM, Bilthoven, the Netherlands). From these samples a 10% stool suspension was made in PBS (Lonza) and subjected to centrifugation (2000 × *g*, 15 min, room temperature (RT)). The supernatant was transferred to a new tube. From this solution tenfold dilutions were made in PBS (Lonza). HAV lysate (HM-175) was cultured in FRhK-4 cells as previously described (Nasser and Metcalf, 1987), although slightly modified. HM-175 quantification was performed by $TCID_{50}$ and an agar overlay plaque assay. Dilutions of this HAV lysate were made in PBS (Lonza). Aliquots of all the spikes were stored at −80 °C until use. Concentrations of genomic copies of all human enteric virus inoculums were determined by real-time RT-PCR.

Water samples were spiked to obtain a final concentration of approx. 6 log GI.4 NoV genomic copies/L, 7 log GII.4 NoV genomic copies/L, 7 log HAV genomic copies/L (estimated final titer of approx. 10^6 $TCID_{50}$/L water sample) and approx. 7 log RV RT-PCRU/L.

2.3. Virus concentration methods

Four viral concentration methods were tested for their efficiency in recovering MNV-1 and MS2 phages in four types of irrigation water (bore hole water, rain water, open well water and river water) and in fresh cut lettuce processing water, all inoculated as stated above. An overview of the viral concentration methods evaluated in this study is presented in Fig. 1. Methods 1, 2 and 3 are based on the virus adsorption and elution (VIRADEL) principle and used an electronegative (HA-filter, Millipore) or an electropositive filter (Zetapor, 3M) (Wyn-Jones and Sellwood, 2001). As viruses are normally negatively charged in the environment, viruses can adsorb to

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