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The concentration of white spot disease virus for its detection in sea water using a combined ferric colloid adsorption- and foam separation-based method

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A B S T R A C T

The white spot disease virus (WSDV), which is most virulent in shrimp, is a cause of serious damage in the shrimp production industry. However, it is difficult to track the infection route and behaviour of WSDV in shrimp farms because it is present at extremely low concentrations in culture sea water. In this study, the concentration of WSDV in sea water foam was examined using dispersed bubbles and milk casein as a surface-active protein. WSDV concentrations were assessed using real-time PCR. When ferric colloid adsorption was performed prior to foam separation, WSDV was effectively removed from sea water and concentrated in the generated foam within 5 min. The removal efficiency was greater than 90% at the optimum iron and casein concentrations of 1 mg Fe/l and 1 mg/l, respectively. Furthermore, to analyse the dissolution of the collected ferric colloid, the WSDV concentration in the colloid-dissolved solution was set to be 200-fold higher than that found in raw water. This represents a novel method of concentrating WSDV for its detection in sea water using a combination of ferric colloid adsorption and foam separation that is easy to perform, rapid and efficient.

1. Introduction

In 2007, the total world shrimp culture production exceeded 3 million tonnes (FAO, 2009). The shrimp aquaculture industry has rapidly developed worldwide, particularly in China, Thailand, Vietnam, Indonesia and India. The most commonly cultured species in the industry is the penaeid shrimp, which has been produced at high levels in tropical and subtropical zones since 1990. However, the production of live Kuruma shrimp (*Marsupenaeus japonicus*) is equally important for fisheries in Japan (Hamasaki and Kitada, 2006). Currently, infections and diseases caused by microbes pathogenic to the cultured shrimp are a menace to shrimp production. The white spot disease virus (WSDV) is the most virulent shrimp pathogen known and causes extensive mortalities of cultured penaeid shrimp species such as *Penaeus monodon* and *M. japonicus.* WSDV affects all of the life stages of *P. monodon* and *M. japonicus* shrimp. It can cause a mortality rate of up to 100% within 3–10 days; thus, it has the potential to inflict substantial economic losses in the shrimp aquaculture industry (Inouye et al., 1994; Takahashi et al., 1994; Chou et al., 1995; Karunasagar et al., 1997). Therefore, the development of effective WSDV countermeasures is a high priority in the shrimp production industry. However, our current knowledge of the mechanisms underlying the infection and accumulation of WSDV is lacking; moreover, there is currently no effective therapeutic tool for treating WSDV infections. The infection spreads through contact with, and predation of, infected shrimp. Furthermore, there is a high risk of transmission via culture sea water. It is difficult to track infection routes and devise quarantine methods for WSDV because there are currently no host cells available for WSDV cultivation, and WSDV is present at extremely low concentrations in the culture sea water. Thus, the detection of WSDV in the culture sea water of shrimp farms is extremely difficult.

In determining the infection routes or behaviours of pathogenic viruses in aquaculture environments, the largest obstacle is the fact that viruses exist at extremely low concentrations in water. Unless determination methods utilising conventional plaque assays or PCR detection are devised, it will remain very difficult to detect viruses in water. For such detection protocols, methods of concentrating trace amounts of viruses are necessary. Several methods for concentrating viruses that are human-related pathogens, such as enteric viruses, have been developed. Currently, the primary method used is membrane adsorption coupled with subsequent elution using a membrane that is either positively (Cliver, 1965; Matsuura et al., 1984; Wyn-Jones et al., 2000; Parshionikar et al., 2003) or negatively (Katayama et al., 2002; Haramoto et al., 2004;

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Rose et al., 2006; Hamza et al., 2009) charged. Using such a membrane adsorption–elution method, substantial amounts of information relating to enteric viruses in drinking water (Haramoto et al., 2004), sewage (Laverick et al., 2004; Lodder and de Roda Husman, 2005; Katayama et al., 2008), river water (Haramoto et al., 2005, 2010; Westrell et al., 2006) and sea water (Katayama et al., 2002; Brooks et al., 2005) have been obtained. However, very little information relating to the concentration and detection of WSDV is available. Membrane filtration is hindered by physical restrictions, such as blockades by suspended solids and colloids. Thus, it is necessary to develop a new method for concentrating WSDV from sea water that is easy to use, rapid and efficient.

Sea foam containing various concentrated bacteria and undefined suspended matter is frequently observed along the water's edge in coastal zones (Southward, 1953). Surface-active substances of biological products, such as polysaccharides (Zhou and Mopper, 1998) and proteins (Maruyama et al., 1991; Chen et al., 1993; Suzuki et al., 2003), appear to play an important role in the concentration of suspended solids in the foam. These surface-active substances not only generate foam on the water surface, but also change the interface of solids from hydrophilic to hydrophobic. Materials with a hydrophobic interface adsorb onto the gas–liquid interface of bubbles. By applying the principle of concentrating suspended solids in sea foam, a foam separation method was developed using dispersed bubbles and surface-active substances (Suzuki and Maruyama, 2002). The primary advantage of this method is the use of proteins or natural surface-active substances as chemical agents for processing. In addition, a technique combining foam separation with coagulation, a solid–liquid separation method for the removal of suspended substances from wastewater, has been developed using ferric chloride as a coagulant and milk casein as a surface-active agent. Therefore, foam separation using dispersed bubbles has a high potential for removing and concentrating pollutants containing proteins and protein-like substances from water into the generated foam. Most viruses are composed of capsid shells containing DNA or RNA, which can be regarded as ultra-fine particles made of proteins. Viral particles seem to adsorb onto surface-active substances such as proteins. Furthermore, viral particles have a strong affinity for ferric colloids and are easy to precipitate though adsorption onto the flocs of ferric coagulants (Zhu et al., 2005).

In this study, the removal and foam-concentration of WSDV from sea water using dispersed bubbles and milk casein as a surface-active protein were tested with and without the pre-adsorption of WSDV onto a ferric colloid. In addition, the sensitivity of WSDV detection in the DNA-extraction liquid of a ferric colloid recovered from the foam was estimated using real-time PCR. To track the behaviours of WSDV for ferric colloid adsorption and foam separation, it is necessary to accurately determine the concentrations of the virus in raw water, treated water and foam water without the recovery loss that is caused by some concentration procedures. In this study, virus-polluted sea water was artificially prepared through the addition of WSDV to determine directly WSDV concentration, and a quantitative evaluation of WSDV was performed in combination with ferric colloid adsorption and foam separation.

2. Materials and methods

2.1. WSDV-polluted sea water

An artificial WSDV challenge was performed by immersing Kuruma shrimp in sea water (150-l glass tank, continuous aeration) with added WSDV (1×10^{10} copies/ml) until infections were confirmed by the appearance of white spots on their bodies. Various organs, including the heart, lymphoid organ, stomach and gills, were removed from the infected shrimp, and mixed tissues (0.2 g) were homogenised with 0.2 ml of physiological saline solution (PSS). The homogenate was suspended in 10 ml of PSS. To purify the suspensions containing the organ homogenates, they were filtered using a cartridge with a fixed glass-fibre filter (25 mm, GF/B type, Whatman, England). The filtrate was then added to 3000 ml of sterilised artificial sea water for the preparation of the WSDV-polluted sea water as raw water.

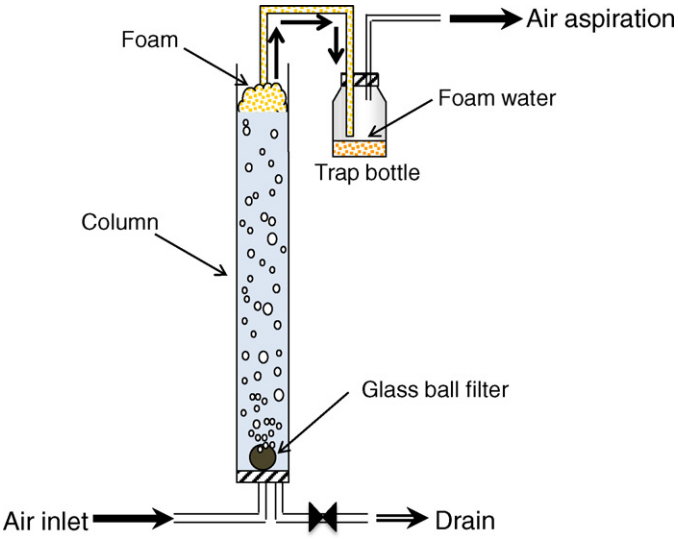

Fig. 1. Schematic diagram of the batch foam separating system (not to scale).

2.2. Foam separation without pre-processing

In previous research using batch equipment (Suzuki and Maruyama, 2002), suspended substances were effectively removed (greater than 99% removal) from polluted sea water by foam separation using 5 mg/l casein. Thus, a concentration of 5 mg/l casein was used in the first trial; subsequently, to determine the optimum concentration of casein, various concentrations ranging from 1 to 5 mg/l were used. An appropriate concentration of milk casein (within the range of 1–5 mg/l), which acts as a surface-active material, was added to each WSDV-polluted sea water sample (180 ml). The stock milk casein solution (reagent grade; Wako Chemical, Japan) was dissolved in 0.01 M NaOH, to a final concentration of 10,000 mg/l. Each sea water sample was stirred rapidly (150 rpm) using a magnetic stirrer for 1 min. Foam separation was performed by transferring samples to the cylindrical column (height, 48 cm; diameter, 2.6 cm) of the batch flotation device (Fig. 1). Dispersed air (mean bubble diameter, 0.6 mm) was supplied from the bottom of the column with a glass-ball filter (pore size range, 5–10 μm; Kinoshita Rika, Japan). Foam generated on the water surface was drawn into a trap bottle using an aspirator. The recovered foam was de-foamed and designated "foam water". The processing time for foam separation was 1 min and the air supply flow rate was 0.3 l of air/min. Samples of the treated water were obtained from the drain. Raw water (WSDV-polluted sea water), treated water and foam water were analysed for WSDV using real-time PCR. The tests were performed in triplicate using identical polluted sea water samples, and the average of the 3 trial values was used as one experimental data point. A series of experiments performed under identical conditions were repeated 3 times using different polluted sea water aliquots from three different containers.