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Geospatial distribution of viromes in tropical freshwater ecosystems

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ABSTRACT

This study seeks to understand the general distribution of virome abundance and diversity in tropical freshwater ecosystems in Singapore and the geospatial distribution of the virome under different landuse patterns. Correlations between diversity, environmental parameters and land use patterns were analyzed and significant correlations were highlighted. Overall, the majority (65.5%) of the annotated virome belonged to bacteriophages. The percentage of *Caudovirales* was higher in reservoirs whereas the percentages of *Dicistroviridae*, *Microviridae* and *Circoviridae* were higher in tributaries. Reservoirs showed a higher Shannon-index virome diversity compared to upstream tributaries. Land use (urbanized, agriculture and parkland areas) influenced the characteristics of the virome distribution pattern. *Dicistroviridae* and *Microviridae* were enriched in urbanized tributaries while *Mimiviridae, Phycodnaviridae*, *Siphoviridae* and *Podoviridae* were enriched in parkland reservoirs. Several sequences closely related to the emerging zoonotic virus, cyclovirus, and the human-related virus (human picobirnavirus), were also detected. In addition, the relative abundance of PMMoV (pepper mild mottle virus) sequences was significantly correlated with RT-qPCR measurements ($0.588 < r < 0.879$, $p < 0.05$). This study shows that spatial factors (e.g., reservoirs/tributaries, land use) are the main drivers of the viral community structure in tropical freshwater ecosystems.

1. Introduction

Viruses are the most abundant living entities on Earth and all living entities are associated with at least one virus which can control microbial communities (Ackermann, 2003). Viral metagenomics have been reported widely in water cycles, especially marine waters, to evaluate the role of viruses in microbial diversity and biogeochemical cycling (Angly et al., 2006; Breitbart et al., 2002; Cassman et al., 2012; Culley et al., 2006).

Determining the factors in constructing the viral community is important for both understanding and manipulating ecosystems (Dinsdale et al., 2008). Factors shaping the viral community in freshwater ecosystems can include temporal factors, geospatial factors, natural disturbances (e.g., typhoon) and human activities (Djikeng et al., 2009; Emerson et al., 2012; Fancello et al., 2013; Ge et al., 2013; Hwang et al., 2017; López-Bueno et al., 2009; Skvortsov et al., 2016; Tseng et al., 2013).

Among all the factors, land use activities is a major factor in shaping waterborne viromes. Land use changes are the primary drivers of the viral community and a range of associated infectious waterborne disease outbreaks (Patz et al. 2004, 2008). In the water cycle, agriculture brings excess nutrients and agricultural chemicals to surface waters, causing oxygen depletion and increasing algal blooms (Foley et al., 2005). Urbanization degrades water quality through surface runoff, and human pathogenic viruses have been detected more frequently in watersheds with dominant urban and agricultural land cover (Corsi et al., 2014; Lenaker et al., 2017). Furthermore, human expansion into wildlife habitats or construction of zoos and animal parks provide opportunities for human-wildlife interactions, thus, increasing the risk for the possible transmission of zoonotic viruses to human populations (Patz et al., 2008). These different anthropogenic activities harbor diverse and distinct viral hosts, including bacteria, plants, wild animals and humans. It is hypothesized that the environment surrounding these reservoirs may favor distinct viral predators and further change the viral community characterization. However, up till now, studies of

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land use impacts on the virome community in freshwater ecosystems are still limited as they mainly rely on traditional methodology (culture-based method or qPCR/RT-qPCR), which focuses on limited human virus targets without considering the whole picture of the viral community in the water environment (Corsi et al., 2014; Lenaker et al., 2017). To date, there is no systematic report focusing on the geospatial distribution and diversity of viromes in natural surface waters and how they may be impacted by human activities and the effect of different land use. In addition, emerging viral pathogens of zoonotic origin could also be discovered through viral metagenomics, for which information is very limited.

Singapore is a highly urbanized island located in the tropics with an area of 719.1 km^2, and a population of 5.61 million in 2016 (Department of Statistics Singapore, 2016). Water is a scarce resource in this country and a total of 17 reservoirs are used to collect rainwater and surface waters for potable water supplies. Increasingly, selected reservoirs are being used as focal points for sporting events (kayaking and dragon boating) and recreational activities so that the public can enjoy and appreciate water resources. As such, good water quality is needed to protect recreational users of these water bodies. Previous studies have detected waterborne viral pathogens (e.g., norovirus GI/GII, adenovirus, rotavirus and astrovirus) in Singapore water bodies using qPCR/RT-qPCR (Aw and Gin, 2011; Aw et al., 2009; Rezaeinejad et al., 2014). Thus, continued surveillance of these viral targets as well as a broader range of viral targets expanded to the virome community coupled with land use information, could provide important data and new dimensions for managing the safety of water resources.

For these reasons, understanding the geospatial distribution of viromes is needed. In this study, seven tropical reservoirs with diverse upstream land use functions (i.e., urbanized, agricultural and parkland areas) were examined. The viral community structure and virome populations specific to each of these environments were systematically investigated together with the characteristics of the watershed. By conducting a complete virome analysis of these freshwater ecosystems (especially viral pathogens and fecal viral indicators), a comprehensive picture of the links between the virome community structure and specific land use activities could be elucidated and thus, used to conduct risk assessment of associated waterborne disease. This information would be important for environmental management at a macroscopic level to protect public health.

Thus, the objectives of this study were to: 1) investigate the overall virome distribution and diversity in diverse freshwater ecosystems (reservoirs/tributaries) in a tropical environment, 2) compare the virome community based on the different land use patterns, 3) assess the extent of human-related pathogenic viruses in surface waters, especially emerging zoonotic and human-related viruses, which may have been undetected before.

2. Materials and methods

2.1. Site description and sample collection

A total of seven reservoirs and three catchments were sampled in Singapore during January (Northeast Monsoon) and April (Inter-Monsoon period), 2015 (Table S1). In total, 19 sampling points were surveyed, comprising of 10 locations in the reservoirs and 9 locations in the tributaries. Only reservoirs 1, 2 and 4 had corresponding tributary sampling points. The study sites were divided into 3 categories based on their geospatial characteristics: urbanized, agricultural and parkland areas (Table 1). Apart from storing water and preventing flood control, some of the reservoirs (i.e., R1-3, 6–7) also catered for recreational activities such as kayaking, dragon boating and water skiing. All the upstream tributary sites were designated as non-recreational areas.

2.2. Measurements of environmental parameters

Physical-chemical parameters, including temperature, pH, turbidity, conductivity, salinity, total dissolved solids (TDS) and dissolved oxygen (DO) were measured on site using a Hanna Meter probe (HI9828 Multiparameter Meter; Hanna Instruments). 24-hour rainfall data were obtained from the Singapore Historical Daily Records (http://www.weather.gov.sg/climate-historical-daily/).

2.3. Viral concentration and nucleic acids extraction

2.3.1. Primary concentration, secondary concentration and viral nucleic acids extraction

30-L water samples were collected from each sampling location in three 10-L carboys. The raw-water sample was immediately transported to the lab and concentrated through a hollow fiber ultrafiltration unit with blocking and elution buffer (Hemoflow Fresenius HF80S, Germany) to a final volume of 600 mL. The hollow fiber ultrafilter was purged with nanopure water for 5 min and pre-treated with 500 mL of blocking solution (0.1 g of NaPP in 1 L of nanopure water) for 15 min. After that, the water sample was recirculated until a final volume of approximately 200–250 mL was reached. An elution step was carried out by recirculating around 300 mL of elution buffer (0.1 g of NaPP, 5 mL of Tween 80, and 10 μl of Antiform in 1 L of nanopure water) for 5 min. Both retentate and eluent were combined to a final volume of 600 mL during primary concentration. 200 mL of primary concentrate was further processed to enrich viral particles in a secondary concentration step through polyethylene glycol (PEG) precipitation (10% PEG 8000 (w/v) and 0.3M NaCl) after pH adjustment (pH = 7.2) (Jaykus et al., 1996). After incubation of the mixture at 4 °C for 18 h followed by 14,000 g for 45 min, the pellet was dissolved in 10 mL of phosphate buffer saline (PBS, pH7.2) with an equal volume of chloroform. After centrifugation at 3000 g for 45 min, the supernatant was filtered through a 0.22 μm sterile syringe and further concentrated to a final volume of 1 mL using a 30 kDa ultracentrifugal filter device (Merck Millipore, Ireland). After primary and secondary concentration, 140 μl of viral nucleic acids (DNA and RNA) was extracted using the QIAamp Viral RNA Mini kit (QIAGEN, Hilden, Germany) and then stored at −80 °C (Saeidi et al., 2017).

2.3.2. Reverse transcription, qPCR/RT-qPCR analysis

In order to quantify the human pathogens in reservoirs and their tributaries using qPCR/RT-qPCR, 14 viral targets were performed to include: (1) Four genotypes of male-specific coliphages (FRNA GI-FRNA GIV); (2) Ten human viral pathogens and (3) One plant viral pathogen/microbial indicator (i.e., PMMoV) (Table S3). The majority of targets belonged to Group IV ssRNA (+) except for adenovirus (group I dsDNA) and rotavirus (group III dsRNA). The details of qPCR/RT-qPCR primers and probes are listed in Table S4. The extracted viral nucleic acids were reverse transcribed using ImProm-II Reverse Transcription System for detecting RNA viruses following manufacturer's instructions (Promega, USA). RT products were stored at −20 °C for later analysis. qPCR/RT-qPCR was carried out in a StepOnePlus Real-Time PCR system (Applied Biosystems, USA) using FastStart Universal Probe Master (Rox) (Roche, Germany) following MIQE guidelines (Bustin et al., 2009).

2.4. Random amplification and sequencing of viromes

The extracted nucleic acids were reverse transcribed and amplified to obtain a sufficient quantity of DNA and cDNA (Wang et al., 2002). To ensure there was no microbial contamination, the negative control was run in 1% agarose gel and ascertained that no DNA gel band was present in the negative control lane. After purification of viral DNA and cDNA, samples were sent to SCELSE