RESEARCH IN PROCESS

Metagenomics to identify viral indicators in the produce chain (MAGIC)

SUMMARY

Detection of human pathogenic viruses in produce or irrigation water currently relies on culture-based methods of bacterial indicators, which frequently fail to correlate with the presence of enteric viruses (e.g., human norovirus and hepatitis A virus). Culture-independent metagenomic approaches (i.e., massive sequencing) provide the highest resolution to analyze species diversity, and will be applied to irrigation water, stools (which may contaminate agricultural and produce handling facilities by food handlers), and produce in order to search for new indicators. Our project goal is to identify viral species that correlate with presence/abundance of human enteric viruses in irrigation waters and produce by using next-generation sequencing techniques (Figure 1). The identification of meaningful viral indicator/s will allow the produce industry to simplify the control of enteric viruses by a simple and rapid procedure to detect and quantify the indicator, which in the short term will be implemented in the produce chain.

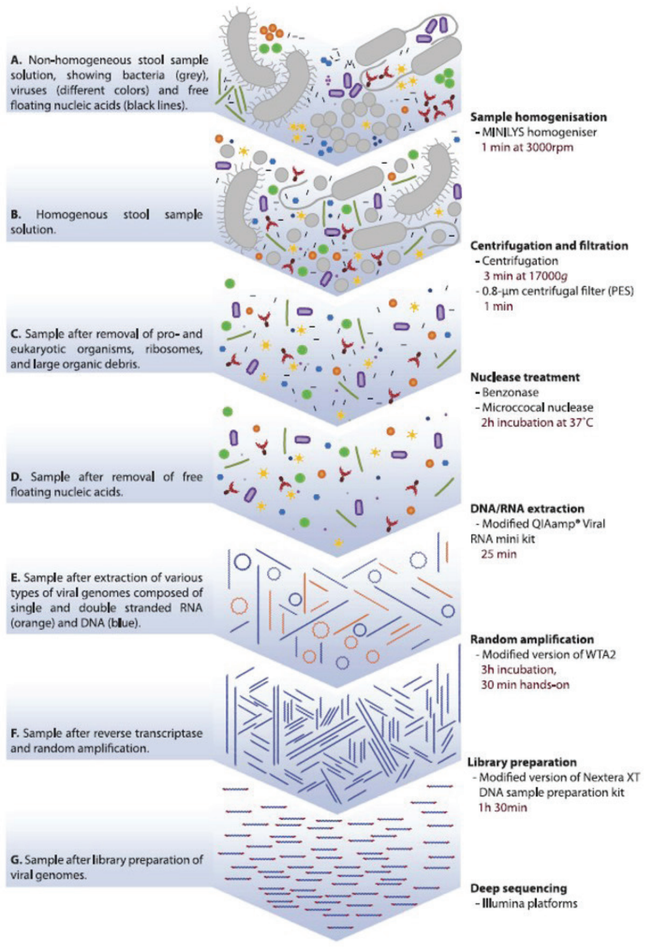
OBJECTIVES

1. Optimization of sample preparation procedure for viral metagenomics from irrigation water samples.
2. Determination of the viral community composition of samples previously analyzed that tested positive or negative for the presence of human pathogenic viruses.
3. Identification of specific viral species or groups whose presence/abundance correlates with the occurrence of human pathogenic viruses in stools, irrigation waters and produce.

METHODS

The work performed during the first trimester focused on Objective 1. Negative irrigation water samples were artificially inoculated with enteric viruses and analyzed according to the NetoVIR procedure (Figure 2), which is able to enrich virus sequences. An external laboratory will construct the next-generation sequencing (NGS) libraries from the DNA and RNA extracted from the irrigation water samples. In parallel, inoculated viruses were quantified by RT-qPCR.

Figure 2. Schematic outline of the NetoVIR protocol (adapted from Conceição-Netto et al., 2015)



RESULTS TO DATE

We evaluated the quantitative effect of homogenization, centrifugation, filtration, and random amplification on a mock-virome (containing human enteric viruses) inoculated in irrigation water samples using RT-qPCR. Overall, RT-qPCR results showed that the NetoVIR protocol is able to recover the inoculated viruses (Table 1). Extracted DNA and RNA of irrigation water samples have been sent to an external laboratory for the construction of the NGS libraries. We are currently comparing two different strategies for library preparation, and we are waiting for the bioinformatics results. Moreover, irrigation water samples positive for norovirus genogroup I (NoV GI), genogroup II (NoV GII), and hepatitis A virus (HAV) have been evaluated with the extraction protocol proposed by the NetoVIR protocol (Table 2).

BENEFITS TO THE INDUSTRY

This research project may significantly contribute to the ongoing efforts to determine a suitable virus indicator/s in irrigation waters and produce. The final outcome of this project will determine the viral community composition of irrigation water samples and produce naturally contaminated with human pathogenic viruses. This information will bridge this scientific technology gap and provide reliable data about the use of phages as indicators of human pathogenic viruses. The project will contribute to the key objectives of the produce industry by a) delineating the viral community that might enter the food chain beyond the recognized pathogenic virus; and b) providing information about the feasibility of phages to indicate human virus contamination. Moreover, information derived from this project will help the produce industry and regulators in decision and policy making, thus enhancing public health.

Figure 1. Schematic outline of the overall project

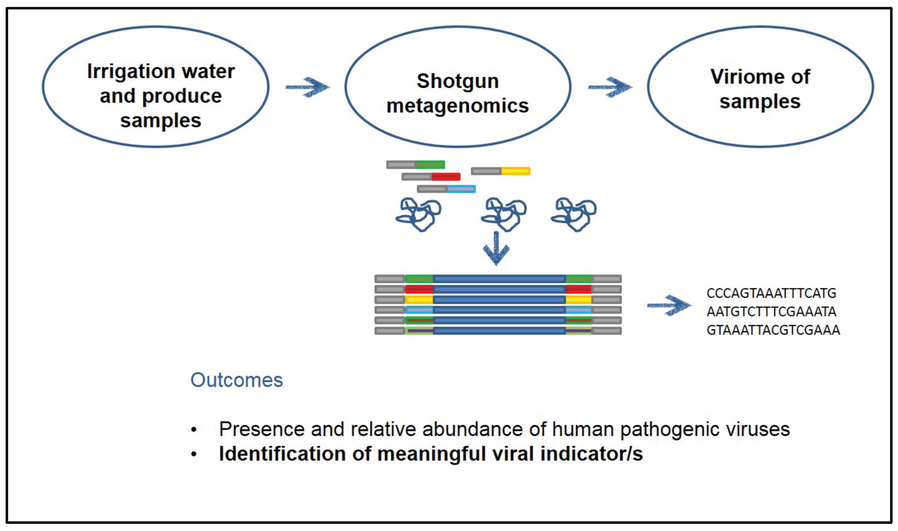


Table 1. Artificially inoculated irrigation water samples processed by standard RNA extraction (control) and by the NetoVIR procedure and quantified by RT-qPCR

	NoV GI	NoV GII	HAV
	log genome copies/ml		
NetoVIR	3.93±0.12	3.83±0.14	6.22±0.01
Control	3.23±0.38	4.87±0.01	6.48 ±0.01

Table 2. Naturally contaminated irrigation water samples processed by standard RNA extraction (control) and by the NetoVIR procedure and quantified by RT-qPCR

		NoV GI	NoV GII
		log genome copies/ml	
Sample 1	NetoVIR	3.41±0.05	3.22±0.12
	Control	3.59±0.22	3.11±0.15
Sample 2	NetoVIR	3.38±0.46	3.30±0.42
	Control	3.05±0.33	3.40±0.20
Sample 3	NetoVIR	4.12±0.16	3.19±0.09
	Control	3.50±0.29	3.72±0.07

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ACKNOWLEDGEMENTS

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LENGTH OF FUNDING

January 1, 2018 – December 31, 2019

CENTER *for* PRODUCE SAFETY