RESEARCH IN PROCESS

Developing Cross-Assembly Phage as a Viral Indicator for Irrigation Waters

SUMMARY

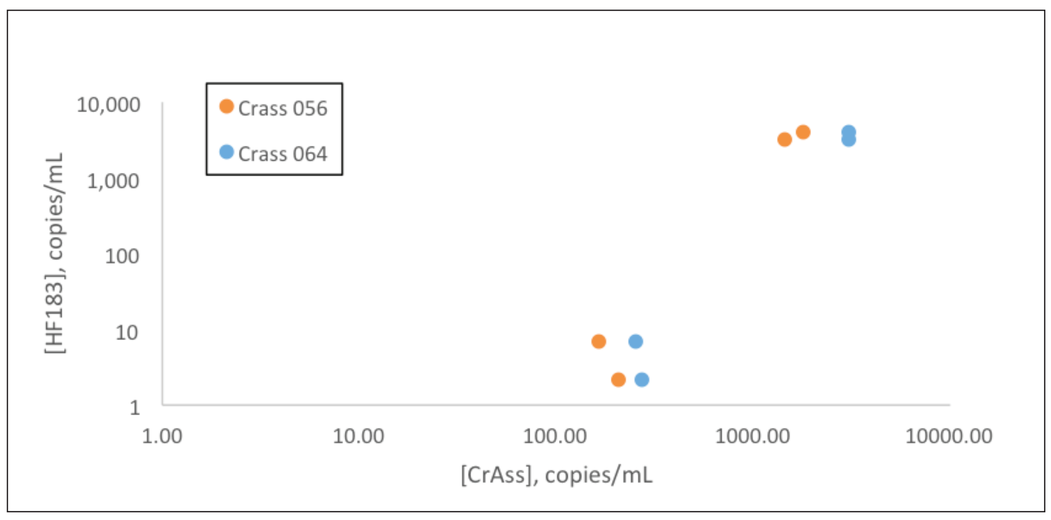
Ensuring high-quality irrigation water is necessary to protect the public when consuming minimally processed produce. The highest risk from exposure to contaminated water is due to viruses; however, water quality is currently monitored using bacteria that are poor representatives of viruses. All previous viral indicators are limited by a low abundance (i.e. difficult to detect) in the environment. Recently, a bacteriophage (virus that infects bacteria), named *cross-assembly phage* (*crAssphage*), was discovered and shown to be more abundant than all other bacteriophages in the human gut combined. Investigations in the PI's research group have shown *crAssphage* to be highly abundant in sewage (Figure below). As *crAssphage* is a virus, it will be a better representative of viral contamination in the environment. In this investigation, we will sample irrigation waters and measure *crAssphage*, viruses, and indicators in these samples to demonstrate the correlation of *crAssphage* and pathogens. We also will determine how much sample volume is necessary to accurately measure *crAssphage*. The development of this viral monitoring tool, catalyzed by funding this project, will enable risk-managers to have an accurate and abundant indicator of viral contamination. This will ultimately provide greater protection of public health and improve consumer confidence in produce consumption.

OBJECTIVES

1. **Collect irrigation water samples.** Irrigation waters will be collected and processed from western Pennsylvania (high sample coverage), as well as Arizona and California (once annual sampling). We will specifically target sampling impacted surface waters utilized for irrigation. Additional regions and sampling sites will be explored for sampling.
2. **Measure and determine correlation of crAssphage, viral pathogen, and FIB levels in irrigation water samples.** Previously developed methods for *crAssphage* detection, viral pathogens, and fecal indicator bacteria (FIB) will be used on collected irrigation water samples. A statistical analysis will then be employed to determine the correlation between *crAssphage*, pathogens, and existing indicators.
3. **Determine *crAssphage* limit of detection.** A critical question in the evaluation of *crAssphage* as a viral indicator for irrigation waters is the amount of sample volume necessary. To address this question, the limit of detection will be determined.

METHODS

Surface irrigation water samples are collected on-site from locations in PA, AZ, and CA and transported on ice for processing. Fecal indicator bacteria and coliphage concentrations, as well as water quality measures, are determined using standard methods. *Cross-assembly phage*, bacterial marker, and viral concentration are determined by qPCR.



RESULTS TO DATE

The project initiated January 2017. To date, samples have been collected from PA, AZ, and CA, and culturable indicators have been measured. Viral concentrate (filters) have been stored and will be analyzed beginning summer 2017.

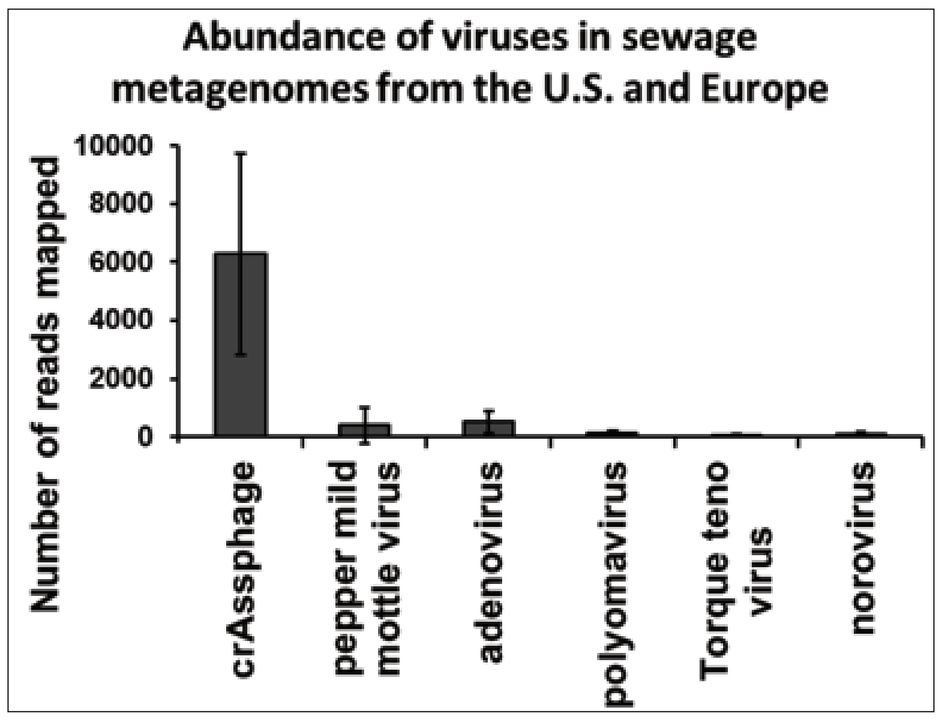
Initial sample evaluation (Figure 1 below) has demonstrated high correlation between the proposed indicator being evaluated (*cross-assembly phage*) and the well-established human source tracking marker HF183.

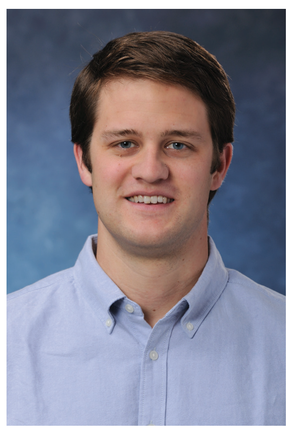
BENEFITS TO THE INDUSTRY

The primary beneficiaries of *crAssphage* development as an abundant viral indicator will be growers and risk-managers. Viral indicators are not currently utilized for risk-based decision-making, and FIB are recognized to be poor indicators of viral contamination, hindering viral-based decision-making. The number of beneficiaries could be as high as the number of growers who currently utilize FIB-based irrigation water monitoring. The development of a viral indicator, which will enable more accurate risk-based decision-making, will improve consumer confidence in the produce and create value for the grower.

Currently, all decisions are made based upon FIB levels, which are known to correlate poorly with viral presence and risk. There is no existing abundant and sewage-specific indicator of viral pollution. Previous development of *crAssphage* for wastewater and recreational water applications suggests its high potential to fill this role in irrigation waters. No previous investigations of *crAssphage* in irrigation waters have been conducted. In the near term, this effort will catalyze further development of the *crAssphage* indicator for direct application in irrigation water monitoring. This work represents a necessary initial development to the application of *crAssphage* as a viral indicator for irrigation water monitoring. Ultimately, this tool will enable risk-based decision-making based upon the presence of a human-specific viral indicator that is more easily measurable than viral pathogens.

Figure 1. Number of reads mapped to various viral genomes from sewage and biosolids metagenomes from the US and Europe (Stachler and Bibby 2014). Number of reads (y-axis) is proportional to the abundance of each measured virus.





CONTACT

Kyle Bibby
University of Pittsburgh
Department of Civil and Environmental Engineering
bibbykj@pitt.edu
412.624.9207

AUTHORS

Christian Ference, Nathalia Aquino, Kyle Bibby

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