Microbial characterization of irrigation waters using rapid, inexpensive, and portable next generation sequencing technologies

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Project funding dates

January 1, 2022 – December 31, 2023

Acknowledgements

The PI and the Co-PIs thank Victoria Obergh, Madison Goforth, Urmi Patel, Caroline Scranton, Jennifer Mydosh and Natalie Brassill for technical assistance on the project, and particularly our industry collaborations on the project: Elston Grubaugh, Malcolm Green, Jeremy Vanderzyl, Nicholas Bahr, Mike Pasquinelli, and Amanda Brooks.

Summary

Pathogen detection approaches utilizing whole genome sequencing (WGS) are being increasingly applied for tracing microbial contaminants, and the produce industry can directly benefit from new methods such as shotgun metagenomics. Shotgun metagenomics allows for the rapid identification of all the bacterial, viral, fungal, and protozoan pathogens in a sample using just one test. WGS technologies are also quickly becoming less expensive and more compact, which could allow for rapid on-site testing during surveillance programs. However, these new technologies must first be verified and validated prior to use. The goal of this project is to investigate two technologies that offer slightly different approaches for pathogen detection, identify the benefits and limitations of each, verify the results, and validate their applications for the produce industry.

Objectives

We hypothesize that shotgun metagenomics without pathogen enrichment culture, using either the Illumina iSeq100 or Oxford Nanopore MinION sequencing technologies, can identify various pathogens alone or in combination in different qualities of agricultural water at the level of <104 cells/ml in a sample.

1. Evaluate the detection limits of the iSeq100 and MinION sequencing technologies for three bacterial pathogens, two viral pathogens, and one protozoan pathogen in agricultural waters of varying quality.
2. Use shotgun metagenomics to characterize the microbial communities of agricultural waters from several Southwest regions using the "gold standard" of large amounts of Illumina sequencing and compare to the portable MinION technology.
3. Conduct whole genome sequencing, shotgun metagenomic, MinION, and iSeq100 workshops/trainings for the produce industry.

Methods

To accomplish Objective 1, experiments will determine the detection limit of the Illumina iSeq100 and MinION technologies by spiking different pathogen concentrations (101–104 CFU/ml) and combinations into agricultural waters of varying quality and conducting shotgun metagenomics using the technologies. To accomplish Objective 2, experiments will characterize the microbial communities of agricultural water from six locations during four different seasonal time points. The communities identified using shotgun metagenomics will be compared between Illumina sequencing ("gold standard") and MinION sequencing for the same samples. The workshops in Objective 3 will: (1) present the results to stakeholders; (2) deliver additional information on WGS, shotgun metagenomics, and how WGS is utilized by regulatory agencies; and (3) provide hands-on training of MinION and iSeq100 technologies.

Results to Date

In total, 192 agricultural water samples have been spiked with different combinations of six foodborne pathogens (**Table 1**) at various concentrations (101–104 cells/ml) and were sequenced using either Oxford Nanopore MinION technologies. Preliminary results indicate all three bacterial pathogens (*Escherichia coli* O157:H7, *Salmonella*, and *Listeria monocytogenes*) can be detected at 101 CFU/ml with the Oxford Nanopore MinION sequencing (**Figure 1**). However, higher levels of turbidity in agricultural water interfered with pathogen detection by lessening the number of pathogen sequence reads, thus reducing the ability to detect pathogens (**Figure 2**). *Cryptosporidium parvum* could only be effectively detected at 103 oocysts/ml (**Figure 3**), while the two viral pathogens (Adenovirus and Hepatitis A virus) could not be detected at all using MinION sequencing.

Benefits to the Industry

This project directly benefits stakeholders who are interested in conducting pathogen detection more rapidly, less expensively, and potentially on-site by providing a powerful tool with novel approaches. By verifying and validating these methods and providing the industry with guidance, it will allow stakeholders to make informed decisions about implementing these technologies into their specific programs. Implementing this cutting-edge technology will ultimately reduce the effort, time, and overall costs of food safety programs by eliminating the need for multiple screening tests for different pathogens. Furthermore, once the technologies have been employed for food safety surveillance programs, they can easily be adapted for plant pathogen surveillance programs to also enhance surveillance against critical plant diseases like *Fusarium* wilt, downy mildew, or powdery mildew.

Table 1. Mixed pathogen spiking protocol for irrigation water

Bacterial Pathogen	Viral Pathogen	Protozoan Pathogen
Salmonella Typhimurium *Escherichia coli O157:H7* *Listeria monocytogenes*	None	None
None	Hepatitis A virus Adenovirus	None
Salmonella Typhimurium *Escherichia coli O157:H7* *Listeria monocytogenes*	None	*Cryptosporidium parvum*
None	Hepatitis A virus Adenovirus	*Cryptosporidium parvum*
Salmonella Typhimurium *Escherichia coli O157:H7* *Listeria monocytogenes*	Hepatitis A virus Adenovirus	None
Salmonella Typhimurium *Escherichia coli O157:H7* *Listeria monocytogenes*	Hepatitis A virus Adenovirus	*Cryptosporidium parvum*
Salmonella Typhimurium	Hepatitis A virus	*Cryptosporidium parvum*
Escherichia coli O157:H7	Adenovirus	*Cryptosporidium parvum*
Escherichia coli O157:H7 *Listeria monocytogenes*	None	None
None	Adenovirus	*Cryptosporidium parvum*

Agricultural water will be spiked with each of the above combinations at concentrations 10^1, 10^2, 10^3, and 10^4 CFU, particles, or oocysts per ml.

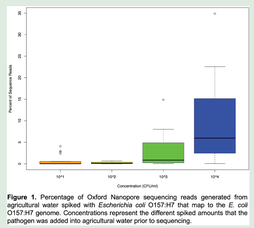

Figure 1. Percentage of Oxford Nanopore sequencing reads generated from agricultural water spiked with *Escherichia coli O157:H7* that map to the *E. coli O157:H7* genome. Concentrations represent the different spiked amounts that the pathogen was added into agricultural water prior to sequencing.

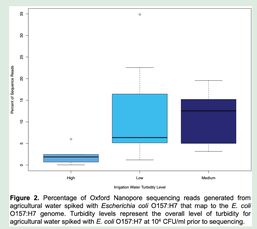

Figure 2. Percentage of Oxford Nanopore sequencing reads generated from agricultural water spiked with *Escherichia coli* O157:H7 that map to the *E. coli* O157:H7 genome. Turbidity levels represent the overall level of turbidity for agricultural water spiked with *E. coli* O157:H7 at 10^4 CFU/ml prior to sequencing.

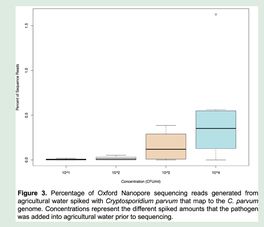

Figure 3. Percentage of Oxford Nanopore sequencing reads generated from agricultural water spiked with *Cryptosporidium parvum* that map to the *C. parvum* genome. Concentrations represent the different spiked amounts that the pathogen was added into agricultural water prior to sequencing.