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

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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 <10^4 cells/ml in a sample. The project has three objectives:

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 (**Figure 1**) by spiking different pathogen concentrations (10^1 – 10^4 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

Preliminary results demonstrated that spiking agricultural water with a combination of *Salmonella* Typhimurium and *Escherichia coli* O157:H7 at high levels (10^6 CFU/ml or 10^7 CFU/ml) and using MinION technology for shotgun metagenomics allowed for identification of the microbial communities including both pathogens. Further analysis allowed us to identify sequence reads specifically associated with the pathogens (**Figure 2**), which allows for genome assembly and further analysis like serotyping, virulence profiling, and source tracking. For example, assembly of the pathogen genomes from the 10^6 CFU/ml spiked sample resulted in the *Salmonella* genome assembling to 93.4% completion and the *E. coli* O157:H7 genome to 85.9% completion. To date, we have conducted all the water spiking experiments, as outlined in **Table 1**, and are currently sequencing the samples.

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.

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.

Table 1. Mixed pathogen spiking protocol for irrigation water

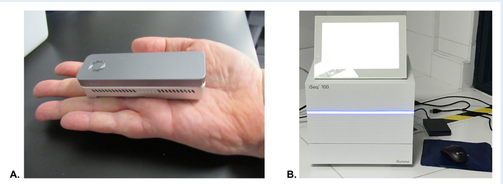

Figure 1. Different sequencing technologies and products used in this project: A) Compact and portable Oxford MinION device in hand, and B) iSeq 100, Illumina's smallest sequencer.

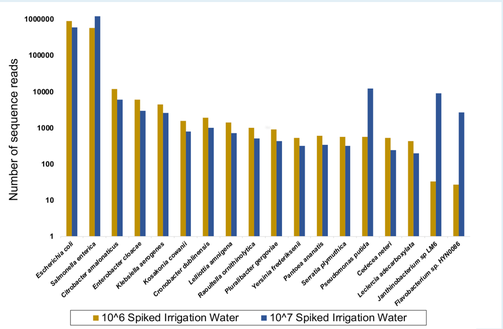

Figure 2. Number of MinION sequencing reads matching different bacterial species in the two agricultural water samples spiked with either 10^6 CFU/ml or 10^7 CFU/ml of *Salmonella* and *E. coli* O157:H7.