Detection of Foodborne Pathogens in Irrigation Water Using a Biosensor: Final Report

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Introduction

Salmonella enterica, *Escherichia coli* O157:H7 and *Listeria monocytogenes* are pathogens frequently involved in foodborne outbreaks and have been isolated from irrigation water. For conventional pathogen detection, cultural methods such as enrichment and plating followed by confirmation are used to detect foodborne pathogens; however, this method often takes 1-2 days, rendering the work and obtaining results long and cumbersome. A biosensor was developed by Phoenix Biometrics to detect airborne bacteria, viruses, yeasts, molds, and spores. The sensor is compact, lightweight, portable and provides "real-time" detection and characterization.

This biosensor is based on over 20 years of research conducted at the Joan B. & Donald R. Diamond Lung Injury Laboratory at the University of Arizona College of Medicine, with continuous support from the U.S. Air Force Office of Scientific Research, for which Dr. Witten served as the principal investigator (Research Professor) prior to retiring from the University of Arizona and advancing to the Phoenix Biometrics Inc. The heart of the biosensor is a modified component of Type II alveolar epithelial cells (a lung surfactant) spun upon a silicon disk. This lipid alveolar epithelial cell simulant biofilm moves in response to the stimulus, in this case the test bacteria. Waves of reflected light intensity and wavelengths off the biofilm are read by a spectrometer. This movement is plotted as mm difference in light intensity vs. nm wavelength. The biosensor works with aerosols, and liquids are tested by nebulizing these liquid suspensions containing the test bacteria, which is how the baseline quantitative curve data was obtained for this project. The objective of this study was to evaluate the efficiency of the biosensor in detecting foodborne pathogens in water.

Methods

Deionized water or irrigation water was inoculated with 5 different bacterial cultures of both foodborne pathogens and surrogate non-pathogenic organisms (*Salmonella*, *E. coli* O157:H7, *L. monocytogenes*, *E. coli* K12 or *L. innocua*) at 5 population levels (ca.10, 100, 1000, 10,000, 100,000 CFU/mL). The water samples were analyzed using the biosensor (Figure 1) per the manufacturer's instructions. The bacterial populations in the water samples were also enumerated using cultural methods. The inoculated water samples were serially diluted as needed and plated on media selective for *Salmonella*, *E. coli* and *Listeria*, respectively. The plates were incubated at 37°C for 24, 24 and 48 h for *Salmonella*, *E. coli* and *Listeria*, respectively. The colonies were counted to determine the population of the bacteria.

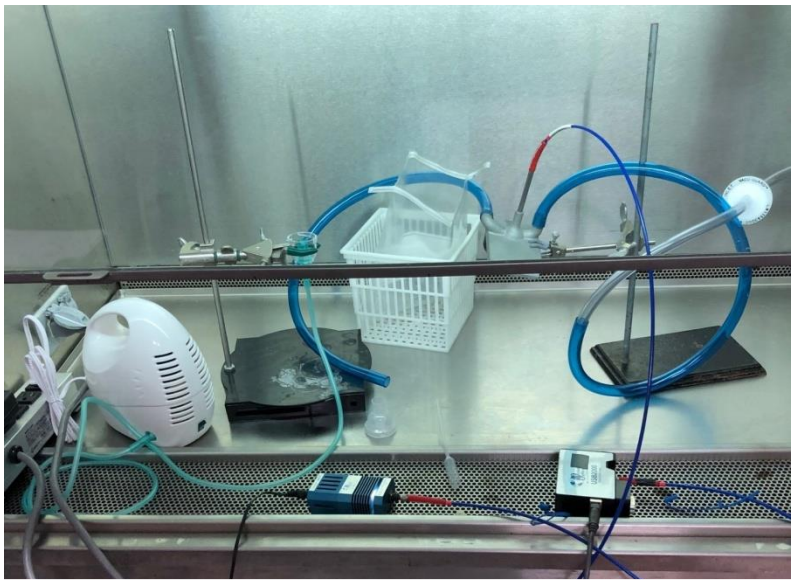

Figure 1. Biosensor developed by Phoenix Biometrics Inc. The water sample was introduced by the nebulizer to the biofilm chamber and detected by the light probe.

Results and Discussions

Five bacterial cultures at various population levels in deionized water or Colorado river irrigation water samples from Yuma were tested and the data is shown in Tables 1-5. The data demonstrates that the biosensor was able to discern a distinct main curve (based on X-axis and Y-axis coordinates with the X-axis in nanometers wavelength and Y-axis in millimeters difference in light intensity). In this project, we used a 300-1200 nanometer light probe to project this intense light on the lipid biofilm to monitor changes in biofilm reflectance from the interaction of the aerosolized bacteria with the biofilm. The biosensor generated separate and different curves for each of these bacteria in deionized water and Colorado river irrigation water from 10 CFU/ml to 10^5 CFU/ml in 0.2 seconds. These bacteria all measured between 620-630 nanometer wavelengths.

The biosensor responded in a much more robust fashion for *E. coli* O157:H7 than *E. coli* K12 as calculated by the slope change of the major peak.

Table 1. Movement of biofilm in response to *Salmonella enterica* as measured by mm difference in light intensity vs. wavelength in nanometers

Salmonella **(Log CFU/ml)**	**In deionized water**		**In irrigation water**	
	X-axis – wavelength in nanometers	**Y-axis – Light intensity in mm**	**X-axis – wavelength in nanometers**	**Y-axis – Light intensity in mm**
1	627.7	4.67	620.0	5.33
2	627.3	3.67	621.7	5.00
3	627.3	3.33	623.0	5.00
4	624.3	3.00	625.7	4.67
5	627.7	3.67	625.3	4.33

Table 2. Movement of biofilm in response to *Escherichia coli* O157:H7 as measured by mm difference in light intensity vs. wavelength in nanometers

E. coli **O157:H7 (Log CFU/ml)**	**In deionized water**		**In irrigation water**	
	X-axis – wavelength in nanometers	**Y-axis – Light intensity in mm**	**X-axis – wavelength in nanometers**	**Y-axis – Light intensity in mm**
1	625.0	6.00	622.7	2.67
2	623.0	5.33	622.7	2.33
3	623.7	5.33	622.7	7.67
4	626.0	6.33	623.3	2.00
5	625.7	5.66	623.2	2.00

Table 3. Movement of biofilm in response to *E. coli K-12* as measured by mm difference in light intensity vs. wavelength in nanometers

E.coli K-12 **(Log CFU/ml)**	**In deionized water**		**In irrigation water**	
	X-axis – wavelength in nanometers	**Y-axis – Light intensity in mm**	**X-axis – wavelength in nanometers**	**Y-axis – Light intensity in mm**
1	633.0	3.33	639.7	3.00
2	630.7	3.50	632.7	4.00
3	630.3	3.70	628.7	6.67

4	627.7	3.00	625.7	9.67
5	631.3	3.00	622.7	6.33

Table 4. Movement of biofilm in response to *Listeria monocytogenes* as measured by mm difference in light intensity vs. wavelength in nanometers

***L.monocytogenes* (Log CFU/ml)**	**In deionized water**		**In irrigation water**	
	X-axis – wavelength in nanometers	**Y-axis – Light intensity in mm**	**X-axis – wavelength in nanometers**	**Y-axis – Light intensity in mm**
1	623.3	2.33	679.0	6.67
2	631.7	3.67	674.7	6.33
3	623.0	4.33	646.0	7.00
4	630.0	3.00	680.0	6.67
5	628.7	3.00	678.7	7.73

Table 5. Movement of biofilm in response to *Listeria innocua* as measured by mm difference in light intensity vs. wavelength in nanometers

***L. innocua* (Log CFU/ml)**	**In deionized water**		**In irrigation water**	
	X-axis – wavelength in nanometers	**Y-axis – Light intensity in mm**	**X-axis – wavelength in nanometers**	**Y-axis – Light intensity in mm**
1	626.7	3.00	624.7	4.33
2	630.3	3.00	622.0	6.00
3	628.7	2.30	625.7	5.67
4	627.3	2.83	623.7	5.00
5	638.7	2.67	625.0	5.33

Additional Research

A grant application, “Real-time biosensor to detect foodborne pathogens in leafy green production” prepared using the preliminary data from this project was submitted and funded (Grant Number SCBGP-HR21-51) by the Arizona Department of Agriculture- Specialty Crop Block Grant Program (SCBGP). This grant focuses on upgrading the prototype and integrating artificial intelligence (AI) to classify the types of bacteria, in addition to concentration prediction, and real-time bacterial detection.

Current research includes successful refinement of the prototype with upgraded optics, capturing wavelengths ranging 617.5 nm to 632.5 nm, and replacing the original filters ranging from 300 – 1,200 nm. This upgraded biosensor generated separate and different curves for *Salmonella*, *E. coli* O157:H7, *E. coli* K12, *L. monocytogenes*, and background microflora including *Pseudomonas fluorescens*, *Lactobacillus*, and yeast at inoculum levels of 10 CFU/ml to 10^6 CFU/ml at 0.025 seconds. Better resolution was demonstrated with a range of 2.0 to 13.0 mm light intensity vs. 623.7 to 627.7 nm wavelength with upgraded optics (compared to previous ranges of 0.93 to 3.33 mm light intensity vs. 622 to 631 nm wavelength), confirming increased accuracy from the upgraded biosensor.

Partnering with AI company Quantiphi of Marlborough, Massachusetts using datasets of *E. coli* O157:H7, *Salmonella*, and *L. monocytogenes* from the upgraded biosensor demonstrated greater classification of bacteria with higher iterations. Increasing the iterations to 100 increased the minimum sensitivity from 78% to 99%, and minimum specificity from 48% to 98%. AI was able to clearly isolate *E. coli* O157:H7 from *Salmonella* and *L. monocytogenes*, with minor overlap between *Salmonella* and *L. monocytogenes*. Current research includes continued machine learning with mixed bacteria and background microflora, as this initial analyses confirms the use cases of AI for classification of type of bacteria, concentration (inoculum levels) prediction, real-time bacterial detection and facility monitoring. Real-time bacterial detection and facility monitoring includes visualization using dashboards with real-time monitoring of multiple sensors at a facility and across facilities, aiding in locating the source and spread of foodborne contamination. In conclusion, a rapid, reliable diagnostic test would help prevent foodborne outbreaks and is critical in maintaining the integrity and sustainability of the US leafy green production systems.