1-1-2014

Characterization Of Bacteriophages From Environmental Water Samples And The Potential Of Bacteriophages Tailspike Proteins (tsp) In Bacteria Detection

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CHARACTERIZATION OF BACTERIOPHAGES FROM ENVIRONMENTAL WATER SAMPLES AND THE POTENTIAL OF BACTERIOPHAGES TAILSPIKE PROTEINS (TSP) IN BACTERIA DETECTION

by

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THESIS

Submitted to the Graduate School

of Wayne State University,

Detroit, Michigan

in partial fulfillment of the requirements

for the degree of

MASTER OF SCIENCE

2014

MAJOR: NUTRITION AND FOOD SCIENCE

Approved By:

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Advisor Date

DEDICATION

I would like to dedicate my Master's Thesis to my advisor Dr. Yifan Zhang who has given me immense depth of knowledge and experience in research in Food Science. I am very thankful to her for allowing me to be a part of her lab and her research.

I would also like to dedicate my thesis to my ever loving parents. Without their support, encouragement, and love, I would never have been so successful and come so far as I have.

ACKNOWLEDGEMENTS

First and foremost, I wish to express my deepest sense of gratitude to my advisor, Dr. Yifan Zhang, for her invaluable guidance and advice given to me to make this research successful. I feel so lucky to have had Dr. Zhang as my advisor and I am really grateful for her patience, motivation, and immense knowledge. Without Dr. Zhang's assistance and contributions, I may not have been able to complete my graduate studies at Wayne State University. All of Dr. Zhang's contributions will be remembered by me always.

In addition to my advisor, I would like to convey my thanks to Dr. Ahmad Heydari and Dr. Kevin Zhou for being kind enough to be a part of the committee, their evaluation of my thesis and their valuable comments.

I am very grateful to Thomas Michael Prychitko for providing me with water samples for the research and the endless assistance, guidance and support given to me to conduct my research, and for helping to write the thesis successfully. I would also like to thank all of my collaborators from other groups and departments at Wayne State University. My special thanks go to Xuebin Tan, Ali Shahini and Dr. Mark Cheng in the Department of Electrical and Computer Engineering and Donna Runft and Dr. Melody Neely in the Department of Immunology and Microbiology at School of Medicine of Wayne State University for the research support related to graphene based biosensor development and phage TSP purification. I also want to sincerely thank Dr. Mike Mei from the Wayne State University Chemistry Department for teaching and helping me with the Transmission Electron Microscopy.

In addition, I would like to thank my fellow labmates in the lab: Liziane da Rocha, Kanika Bhargava, Manisha Polur, Ahmed Hassaballa, Varun Tahlan, Mingyang Huang and Vasiana Tomco for the helpful hands.

I would also like to convey thanks to Dhananjaya Rajasinghe, my husband for his assistance and for giving me generous help throughout the period to make the study a success.

Words cannot convey the immense of debt of gratitude. Finally, I owe to my ever loving parents, my mother Irene Gunathilaka and my father Wilsent Dias Gunathilaka for being with me through all of the hard times and sculpting me into a better person. Your affectionate good wishes always helped me to conduct my work successfully, and your advice, guidance and unbound unconditional love directed me in the right path to step forward in my life. You are the reason for my every success in my life. Words cannot explain how I feel about you and making both of you happy is my sole motivation to achieve anything.

Finally, I pay gratitude to all those who contributed towards the success of this study at least by a word.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

CHAPTER 1

INTRODUCTION

According to the Center for Disease Control and Prevention (CDC), 1 in 6 Americans (about 48 million people) get sick each year along with 250,000 hospitalizations and 3,000 deaths because of the foodborne illnesses (CDC, 2014). Meat, dairy, and poultry products can be contaminated by foodborne pathogens, including *Salmonella*, *Campylobacter*, *Listeria*, and *Escherechia coli* O157:H7, due to the ubiquitous nature of many of these pathogens, and thus may pose serious threat to public health.

The Food and Drug Administration (FDA), the U.S. Department of Agriculture's Food Safety and Inspection Service (FSIS), CDC, and Environmental Protection Agency (EPA) strive to protect food consumers by implementing food safety standards, food laws and regulations, food recalls and investigating food borne outbreaks. However, food safety issues continue to arise, largely due to antibiotic resistance, new and emerging germs, toxins, rising number of multistate outbreaks, changes in food production and supply, changes in the environment leading to food contamination and new and different contaminated foods, such as prepackaged raw cookie dough, bagged spinach, and peanut butter. Adding to the significance of the issue is the low infectious doses of the major foodborne bacteria, which range from one to a few thousands of cells. Previous foodborne outbreak data suggest the infectious dose can be as low as 1 to 10 cells for *E. coli* O157:H7, 15 to 20 cells for some *Salmonella* serovars, and 100 to 1000 cells for *Listeria monocytogenes*. Thus, there is a critical need to develop sensitive and efficient strategy for the detection of bacteria, which allows for early removal of the contaminated samples from the food chain to prevent human foodborne diseases.

1.1 Bacteriophages

Bacteriophages (or phage) are naturally occurring bacterial viruses which infect bacterial cells. They have the ability to multiply inside bacteria and are host specific. Phage are broadly distributed in locations populated by bacterial hosts, such as soil, water and the intestines of animals.

There are an estimated 10^{31} viruses on Earth, most of which are phage. The prevalence of phage changes in different environments and is related to bacterial abundance. Researchers have found phage in a wide range of environments, including the deep sea, alkaline lakes (pH=10), solar salterns (which are ten times saltier than the ocean), acidic hot springs (>80°C with pH=3.0), under >30 m of ice in polar lakes and in the terrestrial subsurface (>2000 m deep) (Breitbart and Rohwer, 2005).

Total phage prevalence across aquatic systems (marine and aquatic systems) typically varies between 10^4 and 10^8 ml^{-1} (Weinbauer, 2004). One of the most dense natural sources for phage is sea water, where up to 9×10^8 virus particles per milliliter have been found, and approximately 70% of aquatic bacteria are infected by phage (Ackermann, 2012).

Phage abundance generally increases with the productivity of the system. For example, in marine systems, it is lowest in the deep sea (10^4–10^5 ml^{-1}), intermediate in offshore surface water (10^5–10^6 ml^{-1}) and highest in coastal environments (10^6–10^7 ml^{-1}) (Paul, 2000). The highest phage number of 9.6 x 10^8 phage ml^{-1} found in an aquatic environment (except sediments) was reported from a cyanobacterial mat (Hennes and Suttle, 1995). In sea ice, phage prevalence was 9.0 x 10^6 – 1.3x10^8 ml^{-1} and is therefore 10–100 times higher than in surrounding water (Maranger et al., 1994).

Phage abundance is typically higher in freshwater than in marine systems. Phage abundance in the surface microlayer, (the first 20 m, of Lake Superior) was 2–15 times higher than in 20 m depth (Tapper, M.A. and Hicks, 1998). In freshwater sediments, phage abundance varied from 0.65 to 2.90 x 10^9 g^{-1}, compared to 0.03 - 1.71 x 10^9 g^{-1} in marine systems (Weinbauer, 2004) and phage abundance typically decreases with the sediment depth. Total abundance of phage in soil and rhizosphere varied between 0.7 and 1.5 x 10^8 g^{-1} (Ashelford et al., 2003).

1.1.1 Morphology and Taxonomy of Phage

Phage are mainly comprised of a nucleic acid molecule (DNA or RNA) surrounded by a protein coat called a capsid. The capsid is comprised of capsomere subunits. The capsomeres consist of a number of protein subunits called protomers. Some phage also contain lipid and other structures such as tails and spikes. They are comprised of double-stranded or single-stranded DNA or RNA (dsDNA, ssDNA, dsRNA, or ssRNA). Their diversity is also reflected by the diversity of genome sizes, which ranges from barely 4 kb to up to 600 kb (a mycobacteriophage) (Brussow and Hendrix, 2002).

Phage are not a homogenous group. The International Committee on Taxonomy of Viruses (ICTV) has classified phage on the basis of their genome (ss versus ds, RNA versus DNA) and their morphology. Currently, 18 families of phage are recognized that infect bacteria and archaea. Of these, one family (*Tectiviridae*) infects both bacteria and archaea, nine families infect bacteria exclusively and eight families infect archaea exclusively. Although archaea possess a few myoviruses and siphoviruses, the demarcation between archaeal and bacterial viruses is generally well defined. Of the viral families with DNA genomes, only two have genomes consisting of single-stranded DNA, while the others genomes consisting of have double

stranded DNA. Eight of the viral families with DNA genomes have circular DNA, while nine have linear DNA. Only five families are enveloped and only two families have RNA genomes. Polyhedral, filamentous, and pleomorphic phage are usually rare and have narrow host ranges (Ackermann, 2012).

Phage are generally categorized by shape as tailed, polyhedral, filamentous and pleomorphic phage.

1. Tailed phage.

They constitute the Order *Caudovirales*, and 96% of all bacterial viruses are tailed phage which come as Families *Myoviridae* (24.5%), *Siphoviridae* (61%), and *Podoviridae* (14%) on the basis of tail morphology (Brussow and Hendrix, 2002) as mentioned in the table1. Phage in the Family *Myoviridae* have long contractile tails while phage in the Families *Siphoviridae*, and *Podoviridae* have long and short noncontractile tails, respectively. The tail of myoviruses consists of an axial needle surrounded by a contractile sheath, separated from the head by an empty space or ‘‘neck.’’ Siphovirus tails are long and flexible or rigid tubes, whereas podovirus tails are short and generally 10–20 nm long. Most tails have fixation structures, such as base plates, fibers, or spikes.

All tailed phage contain linear dsDNA and possess a head and a hollow helical tail built of subunits. The purpose of the tail is to transfer of DNA into a bacterium. The head or capsid is icosahedral and is more or less on elongated derivative of this body. Phage of this type may be virulent or temperate. All are liberated by bursting of an infected bacterium.

Most aquatic viruses are tailed phage of *Myoviridae*, *Siphoviridae*, and *Podoviridae* Families, whereas icosahedral and filamentous phage seem to be rare in water (Ackermann, 2012).

2. Polyhedral phage

They are icosahedral or quasi-icosahedral bodies and they are said to have ‘‘cubic symmetry’’. Phage in the Family *Microviridae* are found not only in enterobacteria, but also in phylogenetically distant hosts such as Bdellovibrio and Chlamydia. Phage in the Family *Corticoviridae* have multilayered capsids of alternating proteins and lipids. Family *Tectiviridae* bacterial viruses contain icosahedral protein capsids which surround lipid-containing vesicles. The latter has the unique property of transforming into a tail-like tube of about 60 nm in length for the purpose of infecting bacteria (Ackermann, 2012). Family *Leviviridae* bacterial viruses are small and resemble the poliovirus. Family *Cystoviridae* bacterial viruses have flexible, lipid-containing envelopes surrounding icosahedral capsid containing three pieces of dsRNA. All polyhedral phage are virulent and are liberated by the bursting of an infected bacterium.

3. Filamentous phage

They comprise Family *Inoviridae* (ssDNA), which include long filaments (Genus Inovirus) or short rods (Genus Plectrovirus) and are probably heterogeneous. Plectroviruses are found in mycoplasmas only. Inoviruses are liberated by slow extrusion from the host bacterium.

4. Pleomorphic phage

They are represented by the Plasmaviridae family. They have lipoprotein envelopes and contain naked dsDNA without a capsid. Plasmaviridae appear as round particles that only infect mycoplasmas and are liberated by budding (Ackermann, 2012).

1.1.2 Transmission Electron Microscopy

Transmission Electron Microscopy (TEM) has enormous importance in the field of virology. Transmission Electron microscopical data are mandatory for classification of a new taxon by the ICTV and TEM has provided a framework for a high-level of virus classification.

The negative staining of isolated phage particles is of universal importance in virology as an easy, fast, and inexpensive approach to the study and identification of viruses by means of a standard transmission electron microscope. Phage particles are dark under negative staining as heads of tailed phage contain a large amount of electron-dense, double-stranded DNA. Tails are pale and much less visible (Ackermann, 2012). Electron microscopy is particularly important for instant analysis and attribution of novel phage to morphospecies, which may or may not be subdivided by molecular criteria.

1.1.3 Host range of phage

Phage are capable of infecting only certain bacteria. Receptor sites (protein molecules) on the surface of bacteria determine the host-specificity of phage. Those receptor sites are recognized by only certain phage, which initiates the infection of bacteria. Those phage receptor sites are positioned in different areas of bacteria. Some of them are located on the cell wall and are present all the time.

The strict host specificity of phage confers high specificity in bacteria detection mediated by phage. In fact, phage has unique advantages in bacteria detection by biosensors. Phage is abundant in the environment, robust to the environmental change, and infects specific bacteria host, which confers it as a great alternative to antibodies in the biosensor application. Phage with broad host range, i.e. being able to propagate in multiple strains or species of bacteria, are good candidates for bacteria detection.

1.2 Bacterial pathogens

The most common foodborne bacterial pathogens are *Salmonella*, *E. coli* O157:H7, *Campylobactor jejuni*, *Clostridium perfringence*, *Bacillus cereus*, *Listeria monocytogenes* and

Staphylococcus aureus. Pathogenic bacteria can contaminate food either by cross contamination with raw products such as meat, juices and produce or by poor personal hygiene. Raw meat, poultry, seafood, and eggs and produce such as lettuce, tomatoes, sprouts, and melons are not sterile and can contain food borne pathogens. Bacteria can also contaminate ready to eat (RTE) meat products during or post processing.

1.2.1 *Salmonella* and *E. coli* spp

Salmonella is a gram-negative, rod-shaped, non-spore-forming facultative anaerobic bacteria. It is predominantly motile and contains flagella that are all around the cell body. They obtain their energy from oxidation and reduction reactions using organic sources and are called chemoorganotrophs. Most subspecies of *Salmonella* produce hydrogen sulfide, which can be easily detected by growing them on media containing ferrous sulfate (Clark and Barret, 1987). *Salmonella* belongs to the same family as *Escherichia*, which has as a species *E. coli*.

The infection with *Salmonella* bacteria is called salmonellosis. *Salmonella* bacteria are the cause of most cases of food poisoning in the U. S. (Mead et al., 1999). Most people who infect with *Salmonella* develop fever, diarrhea, and abdominal cramps 12 to 72 hours after infection. The infection generally lasts 4 to 7 days, and most people recover without treatment (McGhie et al., 2009). However, the diarrhea can be severe for some people which need immediate hospitalization. The *Salmonella* infection can spread from the intestines to the blood stream, and then to other sites in the body in those people and can cause death if the person is not treated with antibiotics immediately.

It is estimated that approximately 42,000 cases of salmonellosis each year in the United States. Approximately 400 deaths are reported every year with acute salmonellosis (Wall et al.,

2010). Many different kinds of *Salmonella* bacteria are exist. The most common *Salmonella* bacteria types in the United States are *Salmonella* serotype Typhimurium and *Salmonella* serotype Enteritidis. *S.* Enteritidis is the most common Salmonella serotype which infect humans in the world (De Lappe et al., 2009). This serotype is more prevalent in poultry and can contaminate eggs from hens which are healthy.

Young children, the elderly, pregnant women and immune-compromised people are prone to have severe *Salmonella* infections. Children who are less than 5 years old have a higher rate of diagnosed infections than the other people.

Escherichia coli are rod-shaped, gram-negative, facultative anaerobic bacteria which are commonly found in the lower intestine of warm-blooded organisms. While most of *E. coli* strains are harmless, some *E. coli* serotypes can cause serious food poisoning, and are responsible for product recalls due to food contamination.

E. coli O157:H7 is the most severe type of *E. coli* and causes bloody diarrhea and can sometimes cause kidney failure or even death. It produces a toxin called Shiga toxin and therefore, it is known as a Shiga toxin-producing *E. coli* (STEC). Hemolytic uremic syndrome (HUS) is a severe complication associated with *E. coli* and the infection produces toxic substances which destroy red blood cells, causing kidney injury. HUS can require kidney dialysis, intensive care, and transfusions.

USDA's Food Safety and Inspection Service (USDA-FSIS) has confirmed *E. coli* O157:H7 in raw ground beef as an adulterant and it is not suitable for consumption. The Topps Meat Company went out of business after 67 years of operation because of a recall of 21. 7×10^6

lb of ground beef contaminated with *E. coli* O157:H7 in 2007 (Nugen and Baeumner, 2008). USDA has zero tolerance regulation policy for seven strains of *E. coli* [*E. coli* O26, O45, O103, O111, O121, and O145] of the pathogen in raw beef products including *E. coli* O157 strain to protect consumers from the dangers of *E. coli* contamination.

1.3 Bacterial detection methods

Traditional bacterial detection methods rely on phenotypic identification using culture, gram staining and biochemical methods, which is very time consuming. Currently, the most commonly used molecular methods are real time Polymerase Chain Reaction (PCR) and microarrays. Real time PCR is highly sensitive and allows bacteria identification at the species level. Microarray based bacterial identification relies on the hybridization of pre-amplified bacterial DNA sequences to arrayed species-specific oligonucleotides. Each probe is tagged with a different colored dye which fluoresces upon hybridization. However, obvious caveats are associated with these methods, such as long time consumed, high cost, expertise involved, and interference from the food ingredients etc.

Biological sensing, utilizing a recognition element and a transduction interface that converts the biological interaction into a measurable output, has great advantages over the above-mentioned methods, including ease of operation, high accuracy and wide detection capacity. Biosensor advancements have greatly improved the ability to detect minute quantities of analytes with very low detection limits. Thirty eight percent of pathogen biosensors have been reported in the past 20 years for the food industry (Huang et al., 2011).

1.4 Use of phage and biosensors for bacterial detection

Phage have been found as a useful tool for rapid detection of pathogens such as *Salmonella* spp., *Mycobacterium tuberculosis*, *Escherichia coli* O157:H7, *Listeria monocytogenes*, and *Staphylococcus aureus* (Goodridge et al., 2003).

Biosensors have great advantages over the traditional methods, including ease of operation, short turnaround, high accuracy, and wide detection capacity. Graphene is a flat monolayer of *sp2* bonded carbon atoms that are packed in a honeycomb lattice. It has demonstrated the great potential in the sensitive measurements of biomoleculars due to its large surface area, high carrier mobility and ohmic electrical contacts (Nguyen 2008; Mannoor 2012). Graphene can be used as a signal transduction material for biosensor development.

Berry's group first reported the graphene based biosensor for achieving single bacteria detection (Mohanty and Berry, 2008). However, their results were based on electrostatic adhesion of the bacteria due to the non-specific attaching, which was not practical for real sensing application. Moreover, their approach lacks the capability to detect different bacteria species. A recent breakthrough shows that the interaction between biomaterials and graphene can be employed as the nanosensor platform for monitoring the bacteria growth on tooth enamel (Mannoor et al., 2012). Bioselective detection of bacteria at single-cell levels was achieved by using the self-assembly antimicrobial peptides on graphene. Particularly, Chen et al, has demonstrated the graphene based biosensor to detect *E. coli* (Huang et al., 2011). In their experiment, (1-pyrenebutanoic acid succinimidyl ester) bio-linker molecule was used to functionalize with CVD graphene and anti-*E. coli* O& K antibody was used for specific binding

with *E. coli*. The results showed that high sensitivity can be achieved by electrical detection with a limit of detection (LOD) of 10 cfu/mL.

Currently, the most common biosensing element for bacteria detection are antibodies (Babacan et al., 2000; Olsen et al., 2003; Pathirana et al., 2000; Skladal, 2003). However, they have many limitations such as the non-antigenic nature of the analyte, incompatibility with the sample matrix or extraction process, and the time and labor intensive process of making antibodies. In addition to the high cost, antibodies are highly fragile and sensitive to environmental conditions. Use of phage for bacteria detection is an emerging technology suitable for real time and inexpensive field detection. Compared to antibodies, phage are less sensitive to environmental stress such as pH and temperature fluctuation and less fragile, which give them a longer field life for detecting bacteria.

The goal of the study

Phage are ubiquitous in the environment and require specific bacteria hosts to grow. Thus, phage with broad host ranges can be used for the detection of bacteria such as *Salmonella* spp. and *Escherichia coli* O157:H7 (Goodridge *et al*, 2003). They also have potential application in monitoring water sanitation. Therefore, the overall goal of this study is to characterize phage from environmental water samples and to develop a novel phage-mediated biosensor for the detection of human pathogenic bacteria in environmental samples**.** The following specific aims have been developed:

Specific Aim I: To isolate phage specific to *Salmonella* and *E. coli* from water samples in the Southeastern Michigan and characterize them by transmission electron microscopy (TEM) and host range determination

Specific aim II: To investigate the potential of phage tailspike protein (TSP) in the detection of *E. coli* O157

CHAPTER 2

METHOD

Specific Aim I: To Isolate and characterize phage specific to human pathogens from environmental water samples

2.1 Water samples

Thirty five water samples were used in this study to isolate phage specific to *Salmonella* and *E. coli* bacteria. Out of the 35 water samples, 2 were collected from a waste water treatment plant (WWTP) and the other 33 water samples were collected from rivers (Deer Creek in Coopersville and Little Pigeon Creek in Agnew) in Southeastern Michigan from June to September 2012. Water samples were frozen at -20°C until processed.

2.2 Bacterial strains

The following indicator strains were used to isolate phage from environmental samples. For *Salmonella* phage isolation, the indicator strains included *S.* Typhimurium ATCC 13311, *S.* Typhimurium ATCC BAA-712, *S.* Typhimurium MZ 1260, *S.* Typhimurium MZ1261, *S.* Typhimurium MZ 1262, *S.* Enteridis MZ 1263. For the isolation of phage specific to *E. coli* 0157:H7, *E. coli* ATCC 700927 was used. All bacterial stock cultures were stored at -80°C in 40% glycerol in Brain heart infusion (BHI) broth.

2.3 Isolation of Phage specific to *Salmonella* and *E. coli* spp. from water samples

Wild type phage were isolated according to a standard protocol described by Wall *et al*, 2010 with some modifications. Briefly, 20 ml of each thawed water sample was centrifuged at 2000g for 10 min and the resulting supernatant was filtered using a 0.2 µm filter, treated with

chloroform (1:100). Five milliliters from each filtered water sample was added to log-phase bacterial cultures of propagation strains in 16.5 ml double strength tryptone soy broth (TSB) and incubated at 37ºC overnight with shaking. The overnight cultures were centrifuged at 1000g for 10 min at 4ºC. The supernatant was filtered through a 0.22 µm filter and treated with chloroform (1:100).

One hundred micro liters of indicator strain was mixed with 4 ml of soft agar (TSB with 0.5% of agar), poured over tryptone soy agar (TSA) plates and allowed to solidify for 5 – 10 minutes. Five to ten microliters of lysate from each filtrate were spotted to the appropriate indicator strain on TSA plates. The TSA plates were incubated for 18 hours at 37ºC and then examined for plaques. Lysis- positive supernatants were purified using a soft agar overlay technique with indicator strains.

2.4 Purification of Phage

A pipette tip was stabbed through the selected plaque and into the hard agar beneath in order to remove an agar plug containing phage. Phage were suspended in 0.5ml of TSB and allowed to incubate at room temperature for 1-2 hours. The suspension was centrifuged at 3220g at 4ºC and the supernatant was transferred to a clean tube. One hundred microliters of phage and 100µl of indicator strain were mixed with 4ml of top agar and the mixture was poured onto a TSA plate. The suspension was mixed well and incubated at 37ºC overnight. Once confluent lysis was observed on the surface of TSA plate after overnight incubation, 2ml of TSB were added to the plate and scraped off the top agar layer. The mixture was centrifuged at 4ºC for 30min at 3220g to get rid of agar and bacteria. The suspension was filtered using 0.2µm filters and a few drops of chloroform were added to the supernatant and stored at 4ºC until use. The

procedure was repeated until uniform size plaques were observed throughout the TSA plate. Once uniform plaques were observed, it was assumed that the phage were purified. Two milliliters of TSB were then added to the plate and scraped off the top agar layer again. The mixture was centrifuged at 4ºC for 30min at 3220g to remove agar and bacteria. The suspension was filtered using 0.2µm filters and a few drops of chloroform were added to the supernatant and stored at -20°C.

2.5 Titration of Phage

Indicator strains were grown in TSB at 37°C with shaking at 230 rpm for 3 hours in order to reach the late log phase. One hundred microliters of indicator strains were mixed with 4ml of soft agar (TSB with 0.5% of agar), poured over TSA plates and allowed to solidify. The TSA plates were divided into quadrants based on a series of serial dilutions of phage lysates that ranged in concentration from 10^{0}-10^{-10}. Ten microliters of lysate were spotted from each dilution tube to the appropriate quadrant on the TSA plates and the plates were incubated at 37°C. The phage titer were calculated using the formula: Plaque Forming Unit (pfu)/ml phage lysate = (plaques/volume plated (ml)) x dilution factor.

2.6 Host range determination

A total of 103 purified phage, including 70 purified *Salmonella* phage and 33 *E. coli* purified phage, were tested for host range determination. A total of 32 bacterial strains, including 14 *E. coli* strains and 18 *Salmonella* strains, were used as indicator strains to test phage lysis ability.

Wild type *E. coli* strains 1-1 A7 (O26:H11), 1-3 A3 (O45:H2), 1-1 C3 (O103:H2), 1-1 D8 (O11:H-), 1-4 B8 (O121:H19), 1-2 B9 (O145:H-), 1-5 D9 (O157:H7), 1-5 F1 (O157:H7), 1-5

E5 (O157:H7), 1-6 A1 (O157:H7) and, standard *E. coli* ATCC 13706, ATCC 23631, ATCC 25922 and *E. coli* O157 ATCC 700927 were used to determine host ranges of *E. coli* phage purified. Wild type *Salmonella* strains 2-7 A1(*S.* Typhimurium), 2-7 A3 (*S.* Anatum), 2-7 A7 (*S.* Muenster), 2-7 B8 (*S.* Enteritidis), 2-7 B9 (*S.* Enteritidis), 2-9 A1 (*S.* Newport), 2-7 C1 (*S.* Cholerasuis), 2-7 A5 *(S.* Typhimurium), 2-7 B2 (*S.* Anatum), 2-7 B6 (*S.* Typhimurium) and standard *S.* Typhimurium ATCC 13311, *S.* Typhimurium ATCC BAA-712, *S.* Typhimurium MZ 1260, *S.* Typhimurium MZ1261, *S.* Typhimurium MZ 1262, *S.* Enteridis MZ 1263 were used to determine host ranges of *Salmonella* phage purified.

Bacterial host ranges of phage were determined by a spot lysis assay according to the method described by Lu et al, 2003. Briefly, 4 ml of soft agar (TSB with 0.5% of agar) were seeded with 100 µl of indicator strains (10^6 cfu/ml), mixed gently and poured onto TSA plates. The top agars were allowed to solidify at room temperature, and 10 µl of a phage suspensions (10^7 pfu/ml – 10^{10} pfu/ml) were pipetted onto the top agar layers. The plates were incubated at 37°C overnight, and then examined for the presence of clear zones of lysis. Clear zones, resulting from the lysis of host cells, indicated the presence of phage. Lytic activities of phage were recorded on a scale as follows: (-) no lysis/plate and (+) lysis/plate.

2.7 Transmission Electron Microscopy (TEM)

Phage which showed broad host ranges were examined by TEM. Prior to TEM, Phage were first sedimented in 0.85 % saline according to the method described by Handa *et al*, 2008 with some modifications. Briefly, 100 µl of indicator strain (10^6 cfu/ml) and 100 µl of phage (titer approximately 10^9 pfu/ml) were mixed with 50 ml of Luria-Bertani (LB) media and incubated overnight at 37°C. The overnight culture was centrifuged at 10, 000 rpm for 20 min to remove uninfected cells and other debris. This solution was then filtered using a 0.22 µm

millipore filter. The filtrate containing the phage was tittered and stored at 4°C until use (Handa et al., 2008). Phage lysate (titer approximately 10^9 PFU/ml) was then ultra centrifuged at 35, 000 rpm in a Beckman SW41-Ti rotor for 2 hours using a 13 ml Beckman ultracentrifuge tube. The pellet was resuspended in 0.85% saline and ultra centrifuged again. The final pellet was resuspended in 200 µl of 0.85% saline and stored at 4°C until use. The concentration of final solution is approximately between 10^9 and 10^{10} pfu/ml.

One drop (10 µl) of phage (10^9-10^{10} pfu/ml) suspension was applied to a surface of a Formvar-carbon-coated copper grid (200 mesh) and allowed to sit for one minute. Excess liquid was drawn off using filter paper and the grid was allowed to air dry. A drop of 1% (wt/vol) phosphotungstic acid (PTA) (pH 6.5) (Fisher Scientific) was applied to the grid surface and the grid was blotted off immediately and allowed to air dry. The grid was reloaded to a JEM-2010 TEM (JEOL USA INC, 11 Dearborn Road, Peabody, MA 01960) at 200 kV accelerating voltage, (with 100 µm condenser aperture, and 60 µm objective aperture,) and examined under 200,000X and 500,000X magnification.

Specific aim II: To investigate the potential of phage tailspike protein (TSP) in the detection of *E. coli* O157

2.8 Preparation of *E. coli* O157

A single colony of *E. coli* O157 ATCC700927 was inoculated in 10 ml of Luria-Bertani (LB) broth and incubated overnight at 37°C to reach late log phase. The Bacterial suspension was centrifuged at 5000 rpm for 5 min and washed twice with 0.85% saline.

2.9 Propagation of phage ΦV10

Phage ΦV10 is an *E. coli* O157-specific phage and encodes a tail spike that specifically recognizes the O157 antigen of *E. coli* O157 (Perry et al., 2009, Scholl et al, 2009).

One hundred microlitres of *E. coli* O157 ATCC 700927 (10^6 cfu/ml) and 100 µl of ΦV10 phage (titer approximately 10^9 pfu/ml) were mixed with 50 ml of LB media and incubated overnight at 37°C. The overnight culture was centrifuged at 10, 000 rpm for 20 min to remove uninfected cells and other debris. The solution was filtered using a 0.22 µm millipore filter. The filtrate containing ΦV10 phage was tittered and stored at 4°C until use (Handa et al., 2008). Phage lysate (titer approximately 10^9 PFU/ml) was then ultra centrifuged at 35,000 rpm in a Beckman SW41-Ti rotor for 2 hours using a 13 ml Beckman ultracentrifuge tube. The pellet was resuspended in 0.85% saline and ultra centrifuged again. The final pellet was resuspended in 200 µl of 0.85% saline and stored at 4°C until use.

2.10 Purification of TSP from phage ΦV10

Purification of TSP from Phage ΦV10 was done by Donna Runft in the Department of Immunology and Microbiology at School of Medicine of Wayne State University. Briefly, the

sequences for the TSP of Φv10 were amplified by PCR using primers designed from the published sequence of Φv10 (NCBI, NC_007804). The amplified DNA was cloned into the pET-SUMO TA cloning vector (Invitrogen). The plasmid construct was then transformed into Invitrogen DE3 chemically competent *E. coli* cells, followed by induction of *E. coli* to express the protein fragment by an addition of 1 mM (isopropyl beta-D-thiogalactoside) (IPTG).

2.11 Device Fabrication

Graphene was used as an electrode in the biosensor device and the Chemical vapor deposition (CVD) method was used to grow the graphene (Li et al., 2009). Graphene was transferred from a copper surface to a silicon substrate, since the biosensor device requires a silicon substrate. Graphene field-effect transistor (FET) devices were fabricated for bacteria detection (Tan et al., 2013).

The sensing of the bacteria was conducted by measuring the change of the electrical conductance (I-V curve shift) after each binding step. When the negatively charged bacteria are attached to the graphene surface, the Dirac point of the graphene is expected to shift to the right (higher positive gate voltages) due to the increased amount of hole doping to the graphene channel. On the other hand, if the bacteria are positively charged, the Dirac point will shift to the left, which indicates that electrons are induced in the graphene.

2.12 Zeta Potential measurement

Zeta potential measurement was performed in order to determine the charge of the bacteria and to predict the shift direction (Gerrad et al., 2013).

2.13 Immobilization of *E. coli* phage TSPs on graphene surface

Introducing hydroxyl groups, amine groups and carboxyl groups to the graphene is called graphene functionalization. Graphene funtionalization was performed to bind phage TSP to the graphene surface as shown in figure 1 (Dutt et al., 2013).

2.14 Device Testing

To measure the binding of the bacteria with the graphene biosensor, a droplet of fresh *E.coli* bacteria with a concentration of 1×10^{9}~1×10^{10} cfu/ml was deposited to cover the graphene sensing channel and to let the bacteria bind to the TSPs. After 30 minutes, non-binding bacteria were washed off by rinsing the device with tap water. The electrical conductance of the device in response to the bacteria binding was measured by an Ag/AgCl reference gate electrode. A semiconductor characterization system (Keithley 4200) was used to measure the transfer curves through the drain and source. Each of the curves was measured in a tap water liquid environment (Huang et al., 2011).

Device fabrication, zeta potential measurement, immobilization of ΦV10 phage TSP on graphene surface and device testing were done by Xuebin Tan in the Department of Electrical and Computer Engineering at Wayne State University.

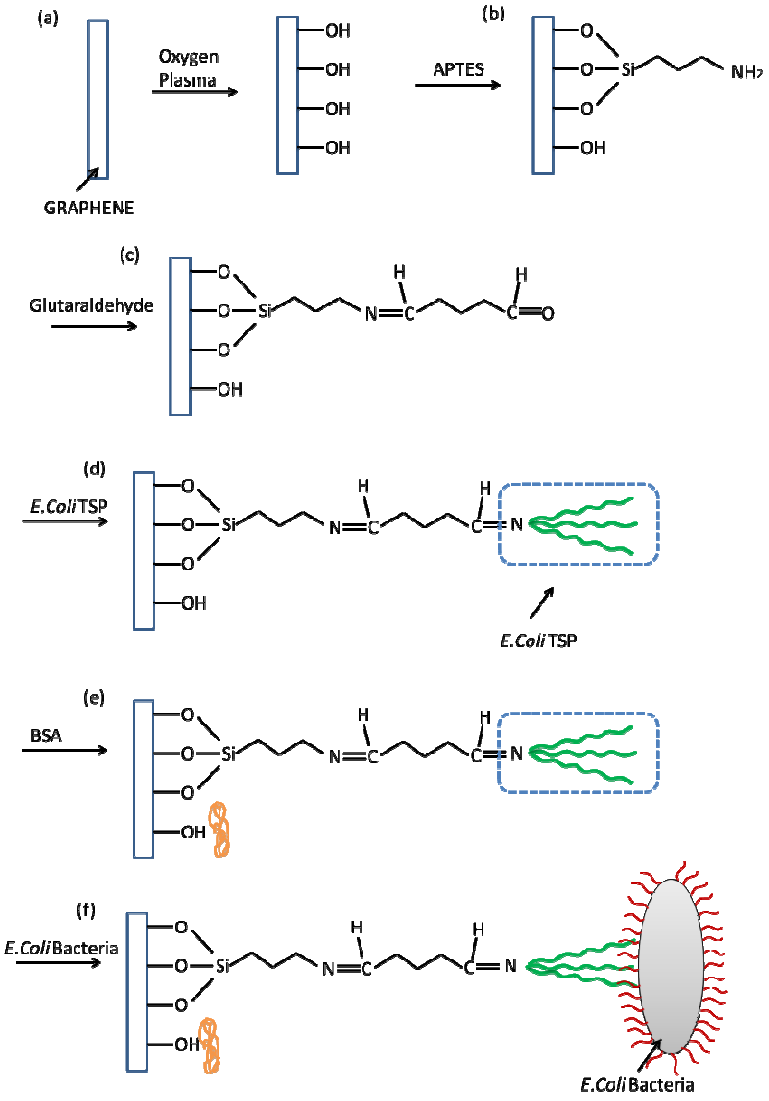

Figure 1: Chemical reaction mechanism of graphene surface functionalization (a) oxygen plasma to introduce the surface oxygen and hydroxyl group, (b) ATPES reaction to introduce the amine group, (c) glutaraldehyde (GA) to introduce the carboxyl group, (d) *E. coli* TSPs immobilization with the carboxyl groups, (e) 1% BSA was applied after step d for blocking the unconjugated sites, (f) *E. coli* bacteria specific binding with *E. coli* TSPs.

(This figure was generated by Xuebin Tan in the Department of Electrical and Computer Engineering at Wayne State University)

CHAPTER 3

RESULTS

Specific Aim I: To Isolate and characterize phage specific to human pathogens from environmental water samples

3.1 Isolation and Purification of phage specific to *Salmonella* and *E. coli* from water samples

Two out of 35 water samples yielded both *E. coli* O157 and *Salmonella* specific phage. None of the river water samples were positive for *E. coli* O157 and *Salmonella* specific phage and all phage isolated were from two WWTP samples.

The two phage samples were able to infect all 6 *Salmonella* indicator strains and *E. coli* O157 indicator strain tested. Therefore, a total of 12 *Salmonella* phage and 2 *E. coli* O157 phage were isolated from two WWTP samples.

Six phage were purified from each phage as 3 large and 3 small phage and, a total of 103 phage were purified. Those 103 phage included 70 purified *Salmonella* phage and 33 *E. coli* phage. Figure 2 shows isolated and purified *Salmonella* phage on lawns of *Salmonella* indicator strains grown on TSA plates.

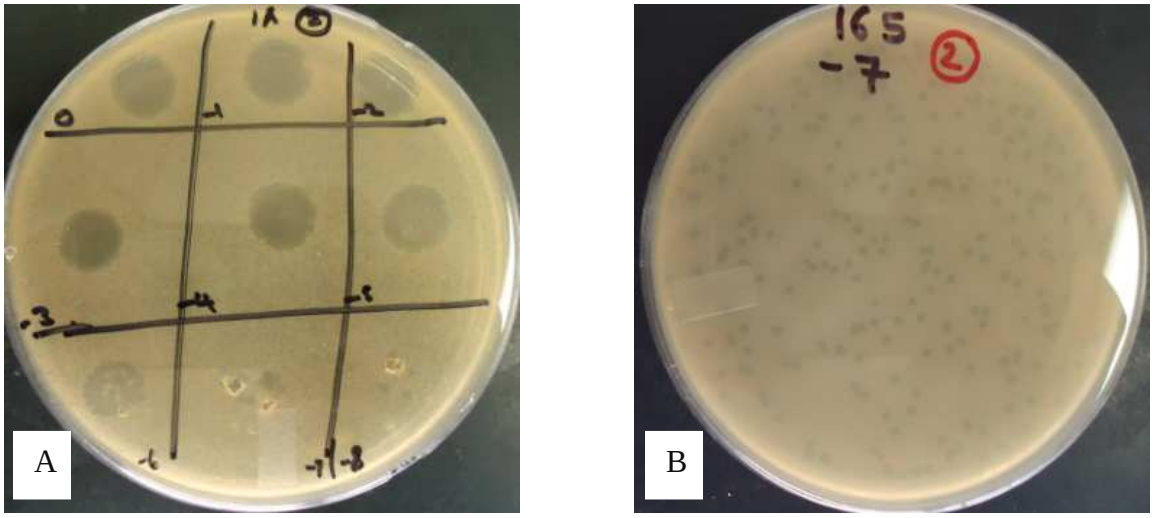

Figure 2. *Salmonella* phage; A: *Salmonella* phage plaques on a TSA plate which has soft agar containing the indicator strain; B: Purified *Salmonella* phage plaques on a TSA plate (soft agar overlay technique)

3.2 Host range determination of phage

Host range was determined for 70 purified *Salmonella* phage using 18 *Salmonella* strains. Thirty three purified *E. coli* phage were tested using 14 *E. coli* strains. Host range of *E. coli* and *Salmonella* phage were summarized as lysis patterns as shown in Table 1 and 2, respectively. Nine phage lysis patterns were identified from a total of 33 *E. coli* phage and 37 phage lysis patterns were identified from a total of 70 *Salmonella* phage.

Out of the *E. coli* phage, coliphages specific to *E. coli* O157 ATCC 700927 exhibited the widest host ranges, while coliphages specific to *E. coli* ATCC 13706 and ATCC 23631 were not able to infect *E. coli* O157 strain ATCC 700927.

Out of 33 *E. coli* phage, 5 phage (PEL1, PEL2, PES1, PES3 and QES1) lysed all *E. coli* strains tested (including all *E. coli* O157 strains) except *E. coli* ATCC 25922 and *E. coli* 0145 wild type strain. They exhibited the widest range which has 85.7% of lysis ability for the *E. coli*

strains tested. Twelve out of 33 phage were able to lyse all *E. coli* O157 strains. Five phage were able to infect all the STEC (shiga toxin producing *E. coli*) serotypes, except *E. coli* O121:H19 (PEL1, PEL2, PES1, PES3 and QES1).

Out of 70 *Salmonella* phage, phage Q167L3 was able to lyse all the *Salmonella* strains tested, and thus exhibited 100% lysis ability for the *Salmonella* strains tested. phage Q167S1 was able to lyse 17 out of 18 *Salmonella* strains (94.4% lysis ability) and another 7 phage were able to lyse 16 out of 18 *Salmonella* strains (88.9% lysis ability). Nine phage were able to infect all 10 *Salmonella* Typhimurium strains tested (P164L1, P164L2, P164L3, P164S3, Q167L1, Q167L2, Q167L3, Q167S2, Q167S3).

Table 1. *E.coli* phage lysis patterns for all *E. coli* strains tested

Pattern	No of phages	Lysis pattern - *E. coli*													
		A	**B**	**C**	**D**	**E**	**F**	**G**	**H**	**I**	**J**	**K**	**L**	**M**	**N**
a	5	+	+	-	+	+	+	+	+	+	+	+	+	-	+
b	4	+	+	-	-	+	-	-	-	-	-	-	-	-	-
c	1	-	+	-	-	+	-	-	-	-	-	-	-	-	-
d	2	+	-	-	-	-	-	-	-	-	-	-	-	-	+
e	2	+	+	-	+	+	+	+	+	+	+	+	-	-	+
f	1	+	+	-	+	+	+	+	+	+	-	+	-	-	-
g	4	+	+	-	+	+	+	+	+	+	+	+	-	-	-
h	3	+	-	-	-	-	-	+	-	-	-	-	-	-	+
i	11	+	+	-	-	-	-	-	-	-	-	-	-	-	-

E. coli strains; **A**: ATCC 13706, **B**: ATCC 23631, **C**: ATCC 25922, *E. coli* O157:H7strains; **D**: ATCC 700927, **E**: 1-5 D9, **F**: 1-5 F1, **G**: 1-5 E5 *E. coli* O26:H11strains; **H**:1-6 A1, **I**:1-1 A7 *E. coli* O45:H2strain; **J**:1-3 A3 *E. coli* O103:H2strain; **K**:1-1 C3 *E. coli* O111:H-strain; **L**: 1-1 D8 *E. coli* O121:H19 strain; **M**:1-4 B8 *E. coli* O145:H-strain; **N**:1-2 B9

Table 2. Selected *Salmonella* phage lysis patterns for all *salmonella* spp tested

pattern	No of phages	Lysis Pattern																	
		A	**B**	**C**	**D**	**E**	**F**	**G**	**H**	**I**	**J**	**K**	**L**	**M**	**N**	**O**	**P**	**Q**	**R**
a	4	+	+	+	+	+	+	+	+	+	+	+	-	+	-	+	+	+	+
b	4	-	-	+	+	+	+	+	+	-	+	-	-	-	-	-	+	+	+
c	2	+	+	+	+	+	-	+	-	-	-	-	+	-	+	+	+	+	+
d	1	+	-	+	+	+	+	+	-	-	+	+	-	-	-	+	+	+	+
e	2	+	+	+	+	+	+	+	+	-	+	-	-	+	-	-	+	+	+
f	1	+	+	+	+	+	-	+	+	-	-	-	+	-	-	+	+	+	+
g	1	+	+	+	+	+	-	+	-	-	+	-	+	-	+	+	+	+	+
h	2	+	+	+	+	+	-	+	-	-	+	-	+	-	-	+	+	+	+
i	1	+	-	+	+	+	+	+	+	-	+	-	+	+	+	+	+	+	+
j	3	+	-	+	+	+	+	+	+	-	+	-	+	+	-	+	+	+	+
k	1	+	+	+	+	+	+	+	+	-	+	-	+	-	-	-	+	-	+
l	1	+	+	+	+	+	+	+	+	-	+	-	-	+	-	-	+	-	+
m	2	+	+	+	+	+	+	+	+	+	+	+	-	-	+	+	+	+	+
n	1	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
o	1	+	+	+	+	+	+	+	+	-	+	+	+	+	+	+	+	+	+
p	1	+	+	+	+	+	+	+	+	+	+	+	+	-	-	+	+	+	+
q	1	+	+	+	+	+	+	+	+	+	+	+	-	-	-	+	+	+	+

Salmonella Typhimurium strains; **A**: ATCC 13311, **B**: ATCC BAA-712, **C**: MZ 1260, **D**: MZ 1261 **E**: MZ 1262, **F**: Salm 213, **G**: ATCC 19585, **H**: 2-7 A1, **I**: 2-7 A5, **J**: 2-7 B6, *Salmonella* Anatum strains; **K**: 2-7 A3, **L**:2-7 B2 *Salmonella* Muenster; **M**: 2-7 A7 *Salmonella* Newport **N**: 2-9 A1 *Salmonella* Cholerasuis **O**: 2-7 C1 *Salmonella* Enteridis **P**: 2-7 B8, **Q**: 2-7 B9, **R**: MZ 1263

3.3 TEM findings

A total of 9 phage (*E. coli* phage: PES1, PEL1, QEL1 and QES1; *Salmonella* phage: tailed P164L1, tailed Q163L2, non-tailed P164L1, non-tailed Q163L2 and Q167L3) which showed broad host range were examined under TEM. Figure 3 shows TEM images of 4 *E. coli* and 5 *Salmonella* phage respectively. Table 3 shows the sizes of the phage (head diameter, tail length and tail diameter) visualized by TEM.

TEM demonstrated that all 4 *E. coli* phage were tailed phage and consisted of icasohedral heads and contractile tails. Tail fibers were visible in all *E. coli* Phage. Heads of the phage varied between 84 and 102 nm in diameter and tail lengths between approximately 113 and 122 nm. Tail diameters varied between 13 and 22 nm. Based on their morphology, all *E. coli* phage belong to the Order *Caudovirales* and the Family *Myoviridae*.

Out of 3 *Salmonella* strains tested, Q167L3 was not a tailed phage and it has 88.89 nm icasohedral head. According to its morphology, it may be belong to the Family *Corticoviridae, Tectiviridae* or *Microviridae*. P164L1 and Q 163L2 consisted of both tailed and non-tailed phage. Tailed phage of P164L1 and Q163L2 have noncontractile and contractile tails respectively. Therefore, based on the morphology of tailed phage of P164L1 and Q163L2, they belong to the Families *Siphoviridae* and *Myoviridae* respectively, in the Order *Caudovirales*. However, the families of non-tailed phage of P164L1 and Q163L2 are unassigned and they may belong to the Family *Corticoviridae, Tectiviridae* or *Microviridae*. Sizes and dimensions of *Salmonella* phage are shown in table 3.

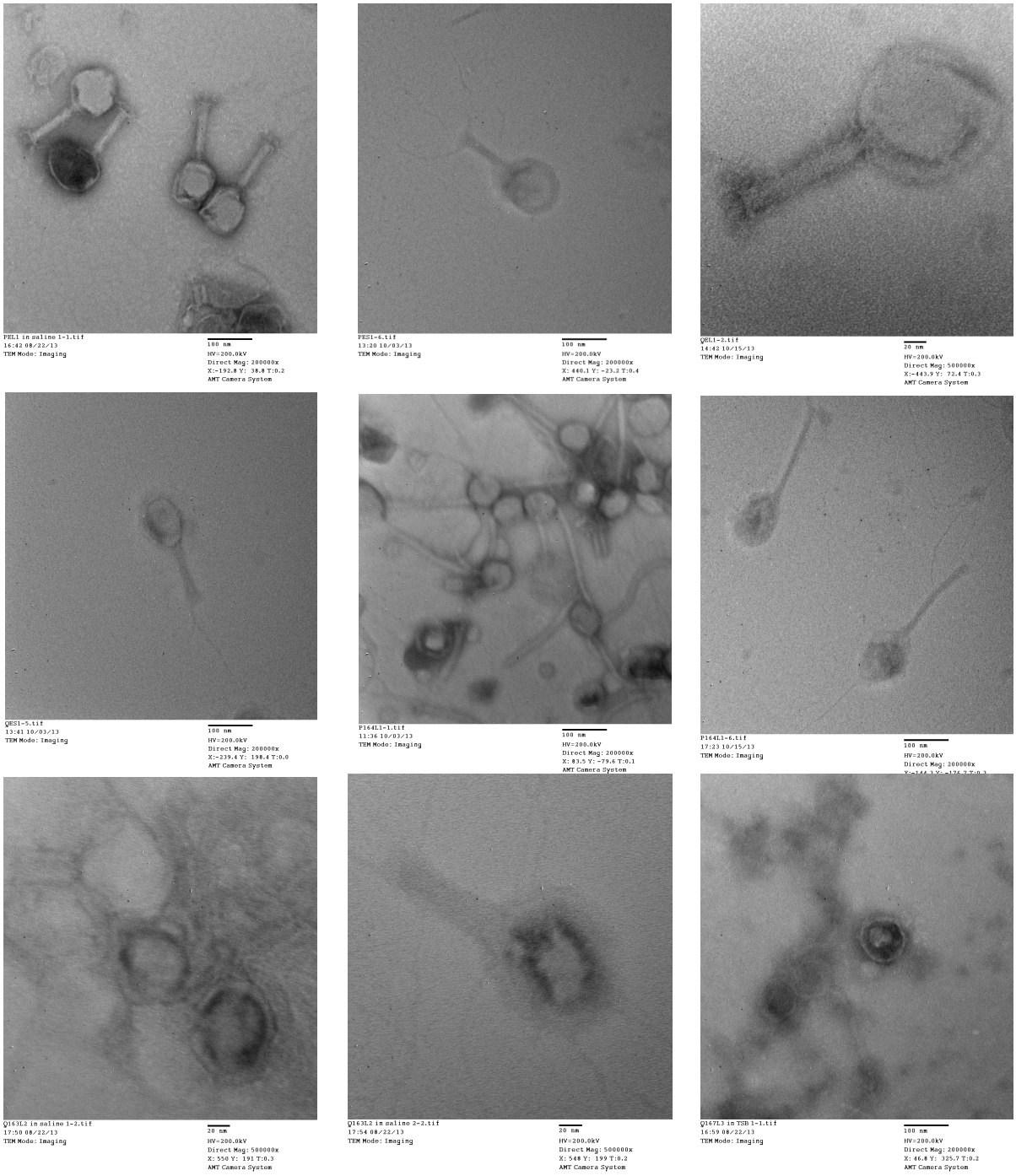

Figure 3. Electron Micrographs of purified *Salmonella* and *E. coli* phage. (1-4: *E. coli* phage [from left] PEL1, PES1, QEL1, QES1; 5-9: *Salmonella* phage [from left] non-tailed P164L1, tailed P164L1, Non-tailed Q163L2, tailed Q163 L2, Q167L3)

Table 3. Morphological characteristics of phage

Phage		Order	Family	Size (nm)		
Type	ID			Head diameter	Tail	
					Diameter	Length
E. coli	PEL1	*Caudovirales*	*Myoviridae*	102.22	20.00	122.22
	PES1	*Caudovirales*	*Myoviridae*	100.00	13.33	117.78
	QEL1	*Caudovirales*	*Myoviridae*	110.00	22.00	120.00
	QES1	*Caudovirales*	*Myoviridae*	84.44	17.78	113.33
Salmonella	P164L1 (non-tailed)	unassigned	Unassigned	75.00	-	-
	P164L1 (tailed)	*Caudovirales*	*Siphoviridae*	73.33	8.89	177.78
	Q163L2 (non-tailed)	unassigned	Unassigned	72.00	-	-
	Q163L2 (tailed)	*Caudovirales*	*Myoviridae*	66.00	20.00	104.00
	Q167L3	unassigned	Unassigned	88.89	20.00	104.00

Specific aim II: To investigate the potential of phage tailspike protein (TSP) in human pathogenic bacteria detection using *E. coli* O157 and phage ΦV10

3.4 Zeta Potential Measurement

The electric properties of *E. coli* were characterized by using the zeta potential, which is the electrical potential between the aqueous solution and the stationary layer of the surrounding liquid attached to the bacteria. Zeta potential of each material (BSA, TSP and bacteria) was measured in tap water at 25 °C and the values are shown in Table 4. The original sample was diluted 10 fold and 100 fold with tap water.

Table 4. Zeta potential of *E. coli* O157, *E. coli* phage (φV10), BSA, and *E. coli* phage TSP in tap water.

Sample Name	Zeta Potential (mV)
E. coli	0.328 ~ 0.517
BSA	-13.1~ -15.8
φV10	-12.2~ -17.3
φV10TSP	-2.88 ~ -15.8

3.5 Device testing

The positively charged bacteria induce extra electrons in the graphene channel. A comprehensive experiment was done using 2 types of bacteria at the same time with graphene FET. Binding to the graphene was only seen in *E. coli* O157 and there was a significant left shift

of the dirac point due to *E. coli* O157. After binding with *E. coli*, the graphene FET was unable to bind any other type of bacteria because of the specificity of this binding.

The binding of *E. coli* O157 to TSP led to a significant left shift of the Dirac point (20 mV) of the graphene FET. The number of *E. coli* O157 binding to the graphene was counted as 33±2 in the 80µm by 50 µm graphene channel area.

A comprehensive specific binding experiment whereby a different type of bacteria (*Salmonella*) was directed toward the *E. coli* phage TSP was conducted. Here the transfer characteristics showed that after *E. coli* O157 binding, there was no significant shift of the Dirac point towards the left.

3.6 Sensitivity of the graphene biosensor with different bacterial counts

Figure 4 summarizes the sensitivity of the graphene bacteria sensors related to the numbers of *E. coli* O157 that specifically bound to the *E. coli* phage TSP. The observed results showed that with a higher population (~50) of bacteria bound to the graphene, the shift of the Dirac point could be as high as 35 mV, which gave sensitivity at 1.24 mV/ bacteria.

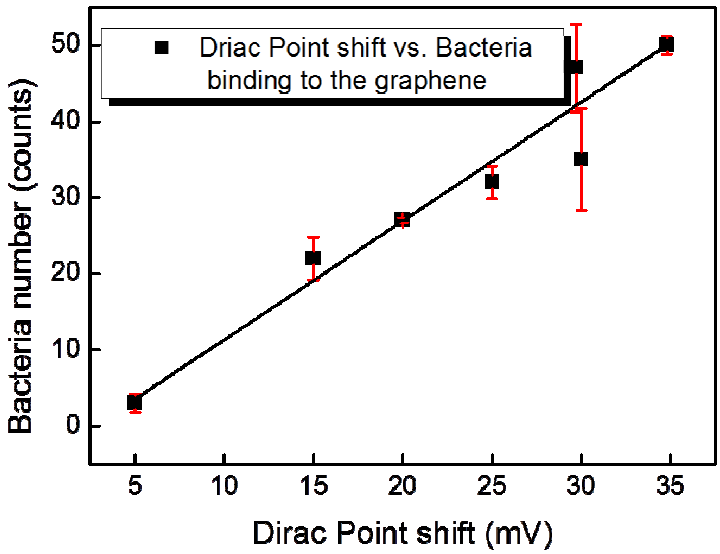

Figure 4. Dirac point shift in response to the number of bacteria binding to the graphene FET. Seven devices were counted for obtaining the statistic information. Linear fitting showed that the sensitivity was 1.24 mV/*E. coli* O157.

CHAPTER 4

DISCUSSION

In this study, we isolated and purified phage specific to *Salmonella* and *E. coli* from water samples. Phage with broad bacterial host ranges are prevalent in water and the high abundance of phage in the aquatic environment made it as an ideal location for phage isolation. Eighteen *Salmonella* and 14 *E. coli* isolates were used in this study to determine host range of *Salmonella* and *E. coli* phage. *Salmonella* Typhimurium and *Salmonella* Enteritidis are the most common *Salmonella* serotypes which infect humans in the United States (De Lappe et al., 2009). Therefore, we included *Salmonella* Typhimurium and *Salmonella* Enteritidis isolates in the study to determine the host range of *Salmonella* phage. There are several public health concerns with *E. coli* and the USDA has zero tolerance regulation policy for Shiga toxin-producing *E. coli* (STEC) which includes *E. coli* O26, O45, O103, O11, O121, O145 and O157. Therefore, in this study, we included seven STEC serogroups for host range determination of *E. coli* phage purified.

Twelve out of 33 *E. coli* phage could lyse more than 50% of *E. coli* isolates tested and 52 out of 70 *Salmonella* phage lysed more than 50% *Salmonella* isolates tested. Twelve *E. coli* phage were able to grow in *E. coli* O157. All *Salmonella* phage comprising of 37 host range patterns were able to multiply in 3 or more indicator strains and 9 phage were able to infect all 10 *S.* Typhimurium strains tested. Out of 9 host range patterns observed in *E. coli* phage, 6 patterns of phage were able to multiply in 3 or more indicator strains and 5 phage were able to infect all the STEC serotypes, except E. coli O121:H19. Therefore, most phage investigated here were able to infect the majority of hosts tested and this suggests a broad host range of the phage

recovered. Many *E. coli* phage were highly effective against strains of STEC O157 and O26 isolated from sources that historically have posed a threat to the food supply (Pearson, 2007). Previous studies have also shown that phage with a broad host range are able to lyse STEC (Viscardi et al., 2007 and Niu et al., 2009) and other pathogenic bacteria. However, most of the coliphages specific to general *E. coli* ATCC 13706 and ATCC 23631 were able to infect only generic *E. coli* strains.

The broad host ranges observed for most of the phage suggest their strong potential for use in microbial detection. The morphology of phage revealed by TEM demonstrated different structural features and dimensions. According to International Committee on Taxonomy of viruses (2000), the *Myoviridae* Family contains many phage which infect members of the Enterobacteriaceae, and this is in agreement with our results. Myoviruses often have wider host ranges (Luhtanen et al., 2014). Similarly, *Salmonella* and *E. coli* phage which belong to the Family *Myoviridae* have the widest host ranges in our study.

In the second part of the study, we used *E. coli* O157 and a standard *E. coli* O157 phage (φV10) TSP to explore their potential in bacteria detection. Phage could be a viable alternative for the detection of bacteria because of their abundance, their specificity to the bacterial host and stability in the environment. Therefore, the use of phage in biosensors is one of the fast-growing areas of research related to food safety (Cademartiri et al., 2009; Singh et al., 2010; Tolba et al., 2010). Phage and phage proteins can be immobilized onto various surfaces and act as a biorecognition element in biosensors. Tail Spike Proteins (TSPs) are the phage proteins that attach to the specific binding sites on the surface of bacteria and determine the specificity of the bacterial host. Each TSP has two terminal domains, the C-terminal domain that binds to the cellular LPS receptor of gram negative bacteria (peptidoglycan in the case of gram positive

bacteria) and the N-terminal domain that can be immobilized to the substrate. Adaptation of the TSPs to improve their affinity will allow optimization of these proteins as detection tools. The immobilization of P22 phage TSPs on silicon substrates has been studied and is being used for selective bacterial detection (Singh et al., 2010). However, the graphene based bacteria sensor based on the binding of TSPs has not been reported so far. This system is more sensitive than many other pathogen detection methods and, requires no chemical or genetic alteration of phage. Further, phage retain their infective capabilities while being bound to the graphene surface. The increasing demand for rapid bacterial pathogen detection techniques has driven the strong interest in phage based detection system.

We tested if graphene FET had specificity using different bacteria (*Salmonella*). The shift of dirac point was negligible (small ~5 mv), since there was no specific interaction between *E. coli* phage TSP and *Salmonella* bacteria due to a mismatch of the biofunctional groups, and non-specific binding of *Salmonella* bacteria was prevented by BSA blocking. In contrast, when *E. coli* O157 was used, a significant left shift of the dirac point of graphene FET was obtained. The left shift is in agreement with the zeta potential measurement of the E. coli bacteria we obtained.

In conclusion, we isolated and characterized phage specific to *Salmonella* and *E. coli* from environmental water samples which showed broad host ranges and were categorized into the families *Myoviridae* and *Siphoviridae*. Phage showing broad host ranges can be potential candidates for bacteria detection. We further demonstrated the potential of phage tailspike protein (TSP) in human pathogenic bacteria detection using *E. coli* O157 and phage ΦV10. We developed a graphene FET that has high specificity and sensitivity toward single pathogenic bacteria detection. The binding of *E. coli* O157 caused detectable conductance change of

graphene FETs while other pathogenic bacteria (such as *Salmonella*) did not bind to the functionalized surface.

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ABSTRACT

CHARACTERIZATION OF BACTERIOPHAGES FROM ENVIRONMENTAL WATER SAMPLES AND THE POTENTIAL OF BACTERIOPHAGES TAILSPIKE PROTEINS (TSP) IN BACTERIA DETECTION

by

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May 2014

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The high abundance of phage in the environment and their specificity with the bacteria host make them an excellent tool for bacteria detection. To characterize phage specific to *Salmonella* and *E. coli* from water samples in the Southeastern Michigan and to develop a novel bacterial detection method using phage, environmental water samples were collected from rivers and waste water treatment plant (WWTP) in Southeastern Michigan. *E. coli* and *Salmonella* phage were isolated from water samples using soft agar overlay technique with multiple indicator strains. Host range of phage was determined using 18 *Salmonella* strains and 14 *E. coli* strains. Transmission Electron Microscopy (TEM) was used to visualize the morphology of representative phage. Tailspike Protein (TSP) purified from *E. coli* O157 phage ΦV10 was functionalized on a graphene field-effect-transister (FET). The specificity of TSP and *E. coli* O157 binding was measured by the shift of Dirac Point. A total of 70 *Salmonella* phage and 33 *E. coli* phage were purified. Twelve *E. coli* phages were able to grow in all 4 *E. coli* O157 strains

tested. Out of 37 host range patterns in *Salmonella* phage, all the patterns were able to multiply in 3 strains and above. Out of 9 host range patterns observed in *E. coli* phage, 6 were able to multiply in 3 strains and above. This suggests a broad host range of the phage recovered. According to the TEM pictures, all of the *E. coli* phages and some of *Salmonella* phages were tailed phages. Detectable conductance change was observed when *E. coli* O157 binds to TSP-functionalized graphene FET whereas very small shift in Dirac Point was caused by *Salmonella* binding, indicating high specificity of *E. coli* O157 interaction with TSP. In conclusion, phage with broad host ranges is prevalent in water. Phage has the potential to be applied to bacteria detection.

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Education:

- 2011-2014 Master's in Nutrition and Food Science, Wayne State University, MI, USA
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Selected Publications:

- Talreja, D., Muraleedharan, C., **Gunathilaka, G**., Zhang, Y., Kaye, K. S., Walia, S. K., and Kumar, A. Virulence Properties of Multidrug Resistant Ocular Isolates of *Acinetobacter baumannii*. Current Eye Research. Accepted, 2014.
- da Rocha , L., **Gunathilaka, G.** and Y. Zhang. Antimicrobial-resistant Listeria species from retail meat in metro Detroit. Journal of Food Protection. 2012, 75(12): 2136-41
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Conference Presentations:

- **Gunathilaka, G**., Tan, X., Ming-Cheng, M., and Zhang, Y. Characterization of phage from environmental water samples and the development of a graphene biosensor using phage for bacteria detection. International Association of Food Protection Annual Meeting 2014; 2014 August 3-6; Indianapolis, IN (Oral Presentation by G. Gunathilaka)
- **Gunathilaka, G**., Polur, M., and Zhang, Y. Bacteriophages Specific to Human Pathogens from Environmental Water Samples. Poster session presented at: International Association of Food Protection Annual Meeting 2013; 2013 July 29-31; Charlotte, NC (Poster Presentation by G. Gunathilaka)
- Rajasinghe, M.H.L.D., **Gunathilaka, G.U**., Perera A.L.T., Fernandopulle, N., Development of In House Laboratory Protocols for Detection of Genetically Modified Food, Poster session presented at: 49th Annual Meeting of the American Society for Cell Biology; 2009 December 07; San Diego, CA.