Mechanisms Associated with Attachment of *Escherichia coli* O157:H7 to Lettuce Surfaces

By

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Mechanisms Associated with Attachment of *Escherichia coli* O157:H7 to Produce Surfaces

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ABSTRACT:

Fresh produce is increasingly associated with foodborne outbreaks. In order to develop effective intervention and measures to reduce microbial risks, it is essential to attain a better understand the mechanisms of attachment of foodborne pathogens to fruits and vegetables. Using lettuce as a model, the attachment of *Escherichia coli* O157:H7 to produce surfaces was studied. Strains expressing various extracellular proteins (curli, O157-antigen, and intimin) known to influence attachment of *E. coli* to intestinal cells were evaluated for their physicochemical properties and ability to adhere to cut edge and whole leaf lettuce. *Escherichia coli* O157:H7 strains included: 0018, 43894 and 43895 (curli producing and non-producing); 86-24 (WT), F-12 (O157-antigen negative), pRFBE (O-antigen replaced on plasmid); and 86-24, 86-24Δ*eae*10 (intimin negative). The eleven strains were surveyed for their hydrophobicity and cell charge using hydrophobic interaction chromatography (HIC) and electrostatic interaction chromatography (ESIC) techniques. Iceberg lettuce squares (2 x 2 cm) were inoculated with *E. coli* O157:H7 strains separately (7.0 log CFU/square) and dried in a laminar flow hood. Lettuce was sampled before (unrinsed) and after being rinsed twice with sterile de-ionized water (rinsed). Strips (2 mm wide) of each cut edge of the lettuce were aseptically removed. Cut-edge and whole-leaf samples were homogenized and spiral plated onto Luria-Bertani agar, supplemented with nalidixic acid (50ppm), to assess levels of bacteria remaining on

the lettuce leaf after rinsing. The rinse steps were not effective in significantly removing bacteria from lettuce ($p \geq 0.05$). Curli-producing and non-producing strains preferentially attached to cut edge versus the whole leaf portions of lettuce ($p \leq 0.05$); however the 86-24 strains showed no preference for attachment. With the exception of 0018 curli-producing and non-producing strains, presence/absence of extracellular proteins surveyed did not influence attachment of *E. coli* O157:H7 to either cut edge or whole leaf lettuce. There was significantly greater attachment of the curli-producing 0018 strain over the curli non-producing 0018 strain to cut and whole lettuce surfaces ($p \leq 0.05$). Production of curli and O-polysaccharide significantly increased ($p \leq 0.05$) the cell's overall hydrophobicity of the cell; however this did not affect attachment ($p \geq 0.05$). The overall cell charge of all strains was negative; however, charge did not affect attachment of *E. coli* O157:H7 to lettuce. The presence of extracellular appendages (curli, O157-antigen, intimin) as well as hydrophobicity and cell charge properties had no affect on attachment of the cell to lettuce.

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DEDICATION:

I would like to dedicate this to my late grandparents, Elwyn and Doris Raiden. Growing up, they were a really important part of my life. They both passed away in late 2002. I know that they would be really proud of me for my accomplishment.

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CHAPTER #1: Introduction

Consumption of fresh produce has increased significantly in the United States over the past two decades. This increase can be attributed to nationwide programs, which promote fruit and vegetable consumption. The “eat 5 to 9 a day” and the “Healthy people 2010” programs are programs promoted by the Department of Health and Human Services (DHHS), National Institutes of Health (NIH), National Cancer Institute (NCI), and Centers for Disease Control and Prevention (CDC). These programs promote fruit and vegetable consumption nationally. Coupled with the increase in fresh produce consumption, a heightened importance to ensure that these products are microbiologically safe for consumers has emerged. Foodborne outbreaks related to produce have increased significantly over the past decade. The CDC estimates that the number of outbreaks per year associated with produce has increased significantly from 0.7% of all outbreaks per year in 1970’s to 6.% in 1990’s (Sivapalasingam et al., 2004). This is thought to be related to the increase in produce consumption. The pathogens that have commonly been associated with such outbreaks include: *Salmonella* spp., *Shigella* spp., and Enterohemorrhagic *Escherichia coli* (EHEC).

Escherichia coli O157:H7 emerged as a foodborne pathogen in 1982 when it was linked to gastrointestinal illness caused by consumption of undercooked beef. Once thought to be primarily a pathogen associated with beef, *E. coli* O157:H7 has been increasingly associated with produce foodborne outbreaks in the past decade. For the years between 1982 and 2002, 21% of all *E. coli* O157:H7 outbreaks were linked to produce (Rangel et al., 2005). Lettuce is the most common vegetable associated with *E. coli* O157:H7 produce outbreaks (34%) (Rangel et al., 2005).

Fresh fruits and vegetables are minimally processed, lacking any thermal processing or treatment steps to reduce bacterial loads. Several steps are being taken to prevent potential contamination. On farm training programs such as Good Agricultural Practices program (GAP's) educate growers and packers on ways to reduce microbial contamination and the importance of sanitation procedures. Additionally, some produce items undergo a "rinse" step in the packing shed. This rinse typically involves fluming commodities through chlorinated wash water. These practices are important, but more steps need to be taken to ensure the safety of produce for consumers.

Produce can become contaminated at any point along the path from farm to fork: during harvesting, post harvest handling, processing, packaging or shipping (Beuchat and Ryu, 1997). One of the most recognized avenues of contamination is through the use of poor quality water during irrigation and processing. Contact with contaminated water may transfer contamination to the plant. Other recognized contamination avenues include the use of improperly treated manure as fertilizer and the presence of cattle, birds and other wild animals in the field. The increase in global trade makes it possible for a wide variety of fresh produce to be available year round. With this convenience comes the possibility that imported foods may not comply with U.S. standards.

Removal of pathogens that may be present on produce surfaces has proven difficult. Research has been conducted to develop rinsing agents and sanitizers that may aid in removal of bacteria from produce surfaces. Such products may be used in packing sheds prior to reaching the consumer market, or as a rinse agent used in the home. While this approach is essential to the future of produce safety, research needs to be conducted to provide a better understanding of the cellular mechanisms behind bacterial attachment.

It is hypothesized that bacteria attach to surfaces in two steps, a “loosely” or reversible attachment, and “tightly” or irreversible (Romantschuk, 1992). Specific extracellular appendages present on the *E. coli* O157:H7 cell may influence whether attachment is reversible or irreversible. If these attachment mechanisms were better understood, effective intervention measures to reduce microbial risk may be developed.

This study analyzed several extracellular appendages that can be expressed by *E. coli* O157:H7 which may influence its ability to attach to produce surfaces. There may be a relationship between specific (curli, O-antigen, intimin) and/or nonspecific adhesion factors (physicochemical) and bacterial attachment to leaf lettuce surfaces. Physicochemical properties (hydrophobicity and cell charge) of *E. coli* O157:H7 strains were evaluated. The cells were surveyed for their ability to attach to cut edge or whole leaf lettuce surfaces. Any correlations between the cells physicochemical characteristics and attachment were also assessed.

Bacterial strains used in these studies were obtained from several researchers. To evaluate differences in attachment associated with curli production, the curli-producing and non-producing phenotypes of 0018 were generously donated by Dr. Larry Beuchat from the University of Georgia, Center for Food Safety. The curli-producing and non-producing phenotypes of 43894 and 43895 were generously donated by Dr. Gaylen Uhlich from the Microbial Food Safety Unit at the Agricultural Research Center in Wyndmoor, Pennsylvannia. These strains have been previously surveyed for their ability to adhere to stainless steel surfaces (Ryu et al., 2004; Ryu and Beuchat, 2005).

For purposes of examining the effect of the O157-antigen on attachment, an O-antigen negative strain was used. Three *E. coli* O157:H7 strains were used: 86-24 (wild-

type), F12 (O antigen negative mutant) and 86-24 pRFBE (containing plasmid with O-antigen gene). These strains were kindly provided by Dr. Philip Tarr, University of Washington School of Medicine and previously characterized (Bilge et al., 1996; Kudva et al, 1999).

Finally, *E. coli* O157:H7 86-24 wild-type and intimin negative 86-24Δ*eae*10 were generously donated by Dr. Nancy Cornick from the Institute of Food Safety and Security, Iowa State University. The strains were previously characterized (Cornick et al., 2002; McKee and O'Brien, 1996).

References:

Beuchat, L.R., and JH. Ryu. 1997. Produce handling and processing practices. *Emerg Infect Dis*. 3:459-465.

Bilge, S. S., J. C. Vary, JR, S. F. Dowell, and P. I. Tarr. 1996. Role of *Escherichia coli* O157:H7 O side chain in adherence and analysis of an rfb locus. *Infect. Immun*. 64:4795-4801.

Cornick, N. A., S. L. Booher, and H. W. Moon. 2002. Intimin facilitates colonization by *Escherichia coli* O157:H7 in adult ruminants. *Infect. Immun*. 70(5):2704-2707.

Kudva, I. T., S. Jelacic, P. I. Tarr, P. Youderian, and C. J. Hovde. 1999. Biocontrol of *Escherichia coli* O157:H7 with O157-specific bacteriophages. *Infect. Immun*. 65:3767-3773.

McKee, M. L., and A. D. O'Brien. 1996. Truncated enterohemorrhagic *Escherichia coli* (EHE) O157:H7 intimin (EaeA) fusion proteins promote adherence of EHEC strains to Hep-2 cells. *Infect. Immun*. 64:2225-2233.

Rangel, J. M., P. H. Sparling, C. Crowe, P. M. Griffin and D. L. Swerdlow. 2005. Epidemiology of *Escherichia coli* O157:H7 outbreaks, United States, 1982-2002. *Emerg. Infect. Dis*. 11:603-609.

Romanschuk, M. 1992. Attachment of plant pathogenic bacteria to plant surfaces. *Ann. Rev. Phytopathol*. 30:225-243.

Ryu J. H., H. Kim, J. F. Frank, and L. R. Beuchat. 2004. Attachment and biofilm formation on stainless steel by *Escherichia coli* O157:H7 as affected by curli production. *Lett. Appl. Microbiol*. 39:359-62.

Ryu, J. H. and L. R. Buechat. 2005. Biofilm formation by *Escherichia coli* O157:H7 on stainless steel: effect of exopolysaccharide and curli production on its resistance to chlorine. *Appl. Environ. Microbiol.* 71:247-54.

Sivapalasingam, S., C. R. Freidman, L. Cohen, and R. V. Tauxe. 2004. Fresh produce: a growing cause of outbreaks of foodborne illness in the United States, 1973 through 1997. *J. Food Prot*. 67:2342-2353.

OBJECTIVES:

Objective 1: To determine how the expression of specific *E. coli* O157:H7 cell extracellular proteins (curli, O157-polysaccharide, intimin) affect the cells attachment to iceberg lettuce.

Objective 2: To determine if *E. coli* O157:H7 cells attach preferentially to cut edge or whole leaf structures of iceberg lettuce and how expression of curli, O-polysaccharide and intimin affect the attachment.

Objective 3: To analyze whether the presence of specific *E. coli* O157:H7 cell extracellular proteins (curli, O157-polysaccharide, intimin) affect the cells physicochemical properties (hydrophobicity and cell charge)

Objective 4: To assess any correlation between the physicochemical properties of *E. coli* O157:H7 and attachment to cut edge and whole leaf iceberg lettuce structures.

CHAPTER #2:

Literature Review:

Enterohemorrhagic *Escherichia coli* O157:H7 was first recognized as a foodborne pathogen when associated with an outbreak of hemorrhagic colitis in 1982 (Jay, 2000). The strain was isolated from patients in Oregon and Michigan exhibiting bloody diarrhea and severe abdominal cramping after eating undercooked hamburger at a fast food restaurant (Jay, 2000). Bloody diarrhea is the most common symptom of hemorrhagic colitis, other symptoms of the disease include fever, vomiting, and nausea. (Jay, 2000). Hemorrhagic colitis caused by *E. coli* O157:H7 was directly linked to hemolytic uremic syndrome (HUS) in 1985 (Jay, 2000). Between 2-7% of infected children with *E. coli* O157:H7 hemorrhagic colitis develop HUS (Jay, 2000). HUS causes hemolytic anemia, and thrombocytopenia followed by acute renal failure (Jay, 2000). Another complication related to hemorrhagic colitis is thrombotic thrombocytopenic purpura (TTP), a condition similar to HUS, except it is associated with brain damage and primarily affects the elderly (Doyle and Cliver, 1990).

Escherichia coli O157:H7 is a non-invasive organism, which produces verotoxin as its primary virulence factor (Doyle and Cliver, 1990). Verotoxins are named for their cytotoxicity to African green monkey kidney cells called Vero cells (Brooks et al., 1998, Meng et al., 2001). The verotoxin produced by *E .coli* O157:H7 is similar to the Shiga toxin (*stx*) produced by *Shigella dysenteriae* type 1 and is referred to as a Shiga-like toxin (SLT), however the two toxins differ antigenically and genetically (Brooks et al, 1998). *Escherichia coli* O157:H7 produces two different types of shiga toxins, Stx1 and Stx2.

CHARACTERISTICS:

Escherichia coli are Gram negative rod shaped bacteria which belong to the family, *Enterobacteriaceae* (Meng et al., 2001). Enterohemorrhagic *E. coli* (EHEC) makes up one of the six different classes of diarrheagenic *E. coli*. The other five recognized classes are enterotoxigenic (ETEC), enteropathogenic (EPEC), enteroinvasive (EIEC), enteroaggretive (EAEC), and diffuse adhering (DAEC) (Meng et al., 2001). Of the EHEC strains, *E. coli* O157:H7 is the most widely known disease causing strain. However, there are several other EHEC strains associated with foodborne hemorrhagic colitis and HUS, including O26:H11, 0111:H8, and O157:NM *(33)*.

The incubation period of illness is typically between 2 and 4 days following ingestion of contaminated food. Symptoms typically resolve within one week, however complications, such as HUS and TTP may arise (Meng et al., 2001). The infectious dose is unknown but believed to be between 0.3 to 15 CFU/gram (Meng et al., 2001). *Escherichia coli* spp. are mesophiles, and growth temperatures range between 15 and 45°C with an optimum of 37°C. (Montville, 2001). A water activity of 0.96 is also necessary for growth. (Jay, 2000).

Escherichia coli O157:H7 differs from other strains of *E. coli* because it fails to ferment D-sorbitol within 24 hours (Willshaw et al., 1997; Meng et al., 2001). Differentiation of *E. coli* O157:H7 is typically done with Sorbitol MacConkey agar, where positive colonies are clear (Hitchens et al., 1995). *Escherichia coli* O157:H7 are also unable to hydrolyze 4-methylumbelliferone glucuronide (MUG) or grow well at temperatures greater than 44.5°C in *E. coli* broth which sets it apart from other strains (Meng et al., 2001). Other unique characteristics of *E. coli* O157:H7 strains include

possession of a rather rare attaching and effacing phenotype, and a 60-MDa plasmid (Meng et al., 2001).

EHEC strains cause disease by adhering to epithelial cells and colonizing the large intestine where they produce toxin (Meng et al., 2002). The verotoxin produced is very destructive to the intestinal lining (Meng et al., 2002). There are three stages of attachment which are: localized adherence, signal transduction, and intimate adherence (Meng et al., 2001). These phenomena will be discussed at greater length later in the section entitled attaching and effacing mechanism.

FOODBORNE OUTBREAKS:

Escherichia coli O157:H7 is one of the foodborne pathogens of greatest interest today. It has been estimated that *E. coli* O157:H7 is responsible for approximately 73,000 cases of hemorrhagic colitis, 2168 hospitalizations and 61 deaths each year in the United States (Mead et al., 1999; Meng et al., 2001). Other EHEC strains account for an additional approximation of 37,740 illnesses, 1048 hospitalizations and 30 deaths annually (Mead et al., 1999; Meng et al., 2001). Outbreaks tend to be seasonal with 86% occurring between May and October (Meng, et al., 2001). Seasonality of illnesses associated with *E. coli* O157:H7 may to be due to greater consumption of ground beef during these months, a greater prevalence of transmission among herds during the summer; or greater rates of poor food handling practices (Meng et al., 2001).

The majority of foodborne outbreaks of hemorrhagic colitis originate from meat products, specifically ground beef. A multi-state outbreak of *E. coli* O157:H7 during the summer of 2002 resulted in 38 confirmed cases; 6 cases of HUS and 1 death (CDC, 2002a). The meat was recalled and accounts for one of the largest recalls (19 million

pounds) of meat in U.S. history (CDC, 2002b). *Escherichia coli* O157:H7 is commonly found among the intestinal flora of cattle which are the primary reservoir (Takeuchi and Frank, 2001a; Fratamico et al., 1996; Woody et al., 2000; Meng et al, 2001). Meat is thought to become contaminated through direct contact with intestinal contents, or manure that is caked on the fur of the animal during slaughter. Since contamination of meat occurs at the surface of the carcass, most cooking will eliminate pathogens on intact meats. A more complete cooking process must be given to ground beef which could contain the bacteria throughout. Beef and dairy cattle are common reservoirs for the bacteria with between 10-25% prevalence in most herds (Meng et al., 2001).

Fresh and minimally processed produce and juice products have been increasingly implicated as vehicles of foodborne illness over the last three decades (Sivapalasingam et al., 2004). Researchers estimate that between 1973 and 1997 outbreaks increased from two per year in the 1970's to seven per year in the 1980s' to sixteen per year in the 1990's (Sivapalasingam et al., 2004). *E. coli* O157:H7 has been increasingly related to produce outbreaks. Rangel et al.(2005) reports that between 1982 and 2002, 21% of all *E. coli* O157:H7 outbreaks were linked to produce (Rangel et al., 2005).

In 1991, an outbreak of *E. coli* O157:H7 associated with unpastuerized apple cider in southeastern Massachusetts resulted in 23 cases (Besser et al., 1993; Meng et al., 2001). Cider was made from unwashed pressed apples, the juice was not pasteurized, and no preservatives were added (Besser et al., 1993). The source of apple contamination was thought to be cattle that grazed in fields adjacent to the orchard, or deer which had been spotted nearby (Besser et al., 1993; Meng et al., 2001). Since this outbreak, there

have been two other outbreaks in which apple cider or juice has been linked to *E. coli* O157:H7 infection (CDC, 1996; 1997b).

Lettuce is the most common vegetable associated with outbreaks, accounting for 34% of the outbreaks (Rangel et al. 2005). There were two lettuce-associated outbreaks in 1995, one in Montana (74 case) and one in Maine (37 cases) (Ackers et al., 1998; Meng et al., 2001). In 1996 an outbreak of *E. coli* O157:H6 (47 cases) occurred in Illinois and Connecticut, where mesclun lettuce was the vehicle of infection (Hilborn et al., 1999; Meng et al., 2001). The source of contamination was thought to be similar to apple cider outbreaks where cattle were grazing close to the crop.

In 1999 alone, there were 5 separate outbreaks where the etiological agent was confirmed to be lettuce or other salad ingredients (CDC, 2002c). These outbreaks resulted in a total of 163 cases of hemorrhagic colitis (CDC, 2002c). Other produce types that have been associated with *E. coli* O157:H7 outbreaks since 1993 include alfalfa sprouts, coleslaw, fruit salad, pea salad, cantaloupe and grapes (CDC, 2002c). Most recently, in 2005, Dole brand pre-packaged lettuce was linked to an outbreak of *E. coli* O157:H7 in Minnesota. There were 23 confirmed cases, with 8 of them needing hospitalization and one suffering from kidney failure. This prompted the company to issue a recall of over 245,000 bagged lettuce and salad mixes. Marler-Clark, a law firm in Seattle, Washington has filed three lawsuits against the company.

The abundance of outbreaks associated with lettuce prompted the Food and Drug Agency (FDA) to release a letter in 2004 to all firms that grow, pack or ship fresh lettuce to make them aware of the health hazards (CFSAN, 2004). The letter urged all companies to re-evaluate their procedures and to refer to the FDA's Guide to Minimize

Microbial Food Safety Hazards for Fruits and Vegetables. Industry guidance is essential to reduce or eliminate the occurrence of such outbreaks. Additionally, research must be preformed in order to create a better understanding behind avenues of contamination.

PRODUCE CONTAMINATION:

Since most fruits and vegetables are not processed extensively, there is a higher risk of bacterial contamination of these products at the consumer level. While most bacteria on the surface of produce are spoilage organisms, there is potential for pathogens to be present. Contamination of produce can occur anywhere along the path from the farm to the consumer's table: during cultivation and harvesting; processing; or distributing (Burnett et al., 2000; Takeuchi and Frank, 2001a; Beuchat and Ryu, 1997). These are typically referred to as either pre-harvest or post harvest sources (Beuchat and Ryu, 1997).

Pre-harvest contamination sources include feces, soil, irrigation water, inadequately composted manure, environmental conditions, wild and domestic animals, insects and human handling (Beuchat and Ryu, 1997). The majority of foodborne outbreaks associated with vegetables are thought to be the result of fecal contamination from human or animal sources (Roberts et al., 1998). *Escherichia coli* species (spp.), *Salmonella* spp., and *Shigella* spp. are pathogens typically found in feces, and commonly implicated in produce outbreaks (Beuchat and Ryu, 1997).

Post-harvest sources of contamination include: humans, harvesting equipment, transport containers, wash and rinse water, animals, and improper temperature storage of the foods (Beuchat and Ryu, 1997). The most notable sources are irrigation water, wash water and poor worker hygiene. These sources can be controlled by using treated water,

by providing workers with adequate bathroom facilities, and by hand washing facility availability.

In order to prevent contamination of produce from such sources, it is essential to ensure that steps are taken to minimize the risk of contamination. The United States has introduced a program for farmers called the Good Agricultural Practices (GAP's) program. This voluntary program provides information to farmers regarding ways to prevent contamination of their product. By taking simple steps during growing and harvesting, the potential for contamination can be drastically reduced.

Another concern associated with produce safety includes the increase in global trade. This makes it possible for a wide variety of fresh produce to be available year round. With this convenience comes the possibility that imported foods may not comply with U.S. Standards. In 2000, the Food and Drug Administration (FDA) conducted a survey to determine the risk that imported produce has on the fresh produce market in the United States. The study found that 4% of imported produce sampled was contaminated with either *Salmonella* or *Shigella* spp. (FDA, 2001). Of the produce sampled, two percent of the lettuce was contaminated (FDA, 2001).

PRODUCE CONSUMPTION:

Over the past two decades consumption of fresh produce has increased worldwide (Tauxe et al., 1997). This can be attributed to many different factors. In 1991 the National Cancer Institute (NCI), along with the Produce for Better Health Foundation (PBH) launched a "five-a-day" campaign encouraging Americans to consume at least five servings of fruits and vegetables per day to reduce cancer and ensure lasting health (NCI, 2003). In launching this campaign, there was a surge of advertisements through

the media stressing health benefits associated with the consumption of fresh fruits and vegetables in the diet (NCI, 2003). Within three years of the implementation of the program, the average adult's consumption of produce increased significantly (NCI, 2003). In 1999, an evaluation of this program showed that produce consumption had increased, from 23 to 26 percent since 1991 (NCI, 2003). It was then recommended that more emphasis be placed on this program. Since it's initiation, the partnership has expanded to include the American Cancer Society (ACS), The Center for Disease Control (CDC), the United States Department of Agriculture (USDA), United Fresh Fruit and Vegetable Association, Produce Marketing Association, and National Alliance for Nutrition and Activity (NCI, 2003). Currently the recommendation has been increased, now recommending that men consume 9, and women 7 fruit and vegetable servings per day. As a result, the consumption of fresh fruits and vegetable should increase even more in the years to come, which makes it essential to address produce safety.

Retail sales of fresh cut produce products accounted for 5.8 billion in sales in 1994, and grew to 8.8 billion in 1998 (Rajkowski and Baldwin, 2003; Hodge, 1995). It is estimated that this may double to 19 billion in the coming years (Rajkowski and Baldwin, 2003; Greenleaf 1999). The consumption of fresh produce increased over 12% between 1987 and 199, from 121.6 to 133.2 lbs per capita (Kaufman et al., 2000).

The geographic sources and distribution of fresh fruits and vegetables has expanded rapidly, including increased global trade of these products (Tauxe et al., 1997). Expanding global trade eliminates the seasonality associated with some produce types and also makes it difficult to track their production. The produce market in the U.S. is also shifting to include fresher cut, minimally processed products, including an increased

number of salad bars in grocery stores and restaurants (USDA, 1997; Tauxe et al., 1997). The consumption of raw or minimally processed vegetables is much greater than the consumption of processed canned or frozen vegetables (Garg et al., 1990). Consumers tend to view fresh produce as being healthier and more convenient than canned produce items. Food service providers find less labor and waste associated with purchasing pre-cut and pre-packaged produce (Hurst and Schuler, 1992).

MINIMALLY PROCESSED PRODUCE:

There are several different ways that consumers may purchase fresh produce in grocery stores. Most produce is purchased in bulk as 'raw' vegetables. These items are harvested and either shipped directly to retail, or retained in storage for a period of time (Roberts et al, 1998). These produce types are typically not "processed" or washed at all before being placed in bags, boxes, or crates and shipped to stores (Roberts et al, 1998). Bagged and 'ready to use' (RTU) fresh produce products are increasing in popularity. These fruits and vegetables are fresh cut for added convenience to the consumer. Fresh cut produce are defined as "fruit or vegetables that have been trimmed, peeled or cut into 100% usable product that are bagged or prepackaged" (Rajkowski and Baldwin, 2003; and IFPA, 2000). Microorganisms can proliferate more readily on fresh cut produce due to greater availability of water and other nutrients (Roberts et al, 1998). It is important to follow proper sanitation practices in the packing plants to prevent contamination or discourage microbial growth. RTU produce are typically examined for quality; peeled, cut or shred; washed; dried; and finally packaged before they are presented for sale (Ahvenainen, R. 2000). The most common RTU items include the bagged salads, lettuce mixes and shredded cabbage.

Ready-to-use products and some of the harder fruits are the only type of fresh produce that may receive an anti-microbial, preservative rinse (Francis et al., 1999). A preservative rinse can aid in reducing the microbial load and enzymatic activity of the product, which decreases potential spoilage of the product (Ahvenainen, R. 2000). Common produce rinses include 50-100-ppm chlorine, 1% citric acid or 1% ascorbic acid (Eckert, 1980; Francis et al., 1999). Rinsing procedures are usually automated, where the produce is flumed in flowing water or bubbling systems. This type of process can present a problem for more delicate produce such as lettuce, where being flumed or sprayed with wash waters can place physical stress on the food (Beuchat et al., 1998).

Extensive research has been done to identify more effective methods for reducing bacterial loads. According to Garg et al. (1990) the rinses described previously have been found to be effective in laboratory research, but not in an industrial setting. As the rinsing solution is recycled (sometimes over days) it can become contaminated with bacteria and fungi (Eckert, 1980). If solution chlorine levels drop, or become ineffective, the bacteria can readily infect the produce through lenticels, stomates, and other injuries. This provides another step along the pathway where contamination can occur (Eckert, 1980).

After rinsing, produce is centrifuged to remove any excess water, and then packaged (Francis et al., 1999). Products that are packaged are usually placed in modified atmospheric packaging to further reduce the risk of pathogen growth (Francis et al., 1999). Modified atmospheric packages utilize different percentages of gases to create an optimum balance of gas inside the package to delay spoilage (Ahvenainen, R. 2000).

The optimal gas ratio is 2-5% carbon dioxide, 2-5% oxygen, with the balance nitrogen (Ahvenainen, R. 2000).

ICEBURG LETTUCE:

Lettuce comes from the family Compositae, *Latuca sativa* L. (Deshpande et al., 1998). Lettuce is the most popular salad crop in the United States with its commercial value only exceeded by potatoes (Deshpande et al., 1998). The United States is the second largest lettuce producer in the world, behind China, with California and Arizona as the main lettuce producing states (Boriss and Brunke, 2005). Lettuces are also routinely imported from Mexico.

In the past 25 years, consumption of leaf lettuces has more than doubled. This can be attributed to the growing popularity of the RTU packaged salad mixes. These mixes are primarily made up of leaf varieties of lettuce. In 2001 it was estimated that 34.5 pounds of lettuce per capita was consumed (Boriss and Brunke,2005). Between 1993 and 1999 sales of bagged salads and lettuce mixes increased 560% from 197 million in sales to 1.3 billion (Borris and Brunke, 2005). Of all horticulture food crops, lettuce is one of the few which is rarely processed, and sold exclusively as a fresh raw product (Deshpande et al., 1998).

Lettuces are mainly composed of water, giving microorganisms an environment to thrive. A study completed by Li et al. (2001) found that when cut lettuce was inoculated with 3.2 log CFU/ml of *E. coli* O157:H7 and stored at refrigeration temperatures for 7 days, growth occurred to reach levels of approximately 6 log CFU/ml. This demonstrates that bacteria can receive required growth nutrients from produce and grow during refrigeration. The leakage of fluids from lettuce as a result of transportation

and storage practices could provide nutrients to support growth of bacteria (Li et al., 2001).

BACTERIAL ATTACHMENT:

The best method for decontamination of fresh produce is prevention of exposure to bacteria; however, due to growth methods, this approach is unpractical (Takeuchi and Frank 2001a). Produce, as mentioned previously, is unintentionally contaminated due to the unavoidable presence of bacteria in the environment. Since prevention may be impossible, a better understanding of mechanisms behind the attachment of foodborne pathogens to produce surfaces is essential in developing effective removal methods. Liao and Sapers (2000) agree stating that: "Further investigation on the mechanism as to how bacteria become attached to apple fruits and how attached bacteria become resistant to, or are protected from the sanitizer treatments would lead to the development of more effective methods for cleaning and decontaminating apple fruits destined for juice production, fresh cut products, or fresh consumption". An understanding of attachment mechanisms may aid in the development of tools for effective prevention or decontamination of pathogens from surfaces. Most studies regarding bacterial contamination of produce involve looking for effective rinses or sanitizers to remove the bacteria; however few have considered means of attachment.

Food microbiology research involving produce is very difficult. Numerous factors must be considered including: storage time, temperature, and shelf life. All of these factors may influence bacterial attachment and survival. Takeuchi et al. (2001d) discovered that temperature influenced attachment and penetration of *E. coli* O157:H7 to leaf lettuce when incubated at modified atmosphere conditions. The highest bacterial

attachment level was seen when incubated at 22°C. There were no significant differences in attachment of *E. coli* O157:7 to lettuce incubated at 4, 10 and 37° (Takeuchi et al., 2001d). In a study involving stainless steel processing surfaces, Farrell et al. (1998) demonstrated that *E. coli* O157:H7 can adhere to processing surfaces after only 5 minutes of contact. The bacteria were able to survive for up to 8 days at 4°C at varying humidity (Farrell et al., 1998). These studies indicate that there may be a correlation between attachment time and temperature.

The most studied mechanisms for bacterial attachment involve attachment to various cell cultures. There is ongoing research regarding how *E. coli* O157:H7 adheres to the intestines and causes disease. Epithelial attachment of various other pathogenic *E. coli* strains has been shown to be mediated by various cell surface antigens (Sherman et al, 1987). Some of these antigens include pili, fibrillae, outer membrane proteins, capsular polysaccharides, and lipopolysaccharides (Sherman et al, 1987). However, the specific surface antigens mediating attachment of *E. coli* O157:H7 are less understood and the research results are conflicting (Sherman et al, 1987). Some of the *E. coli* O157:H7 surface antigens that have been researched include: lipopolysaccharide surface antigens; outer membrane proteins; various types of fimbriae/pili; the presence of a 60 mega-dalton plasmid; and the attaching and effacing protein, intimin. There is limited research considering attachment to other surfaces. The most logical starting point in establishing attachment mechanisms is to begin with those appendages which have been previously shown to affect adhesion to cell cultures.

Bacterial attachment is thought to be the first step in contamination of food products. If attachment did not occur, then an environment favorable to bacterial growth

would never become established (Cabedo et al., 1997). Attachment between the microorganism and the plant is thought to occur in two phases. There is an initial attachment that is reversible and may be related to unspecific factors, followed by highly specific irreversible attachment (Romantschuk, 1992). Many food microbiology and food safety studies have begun to distinguish between loosely and tightly bound organisms in the literature (Cabedo et al., 1996; Tacheuchi and Frank, 2001c).

An important step in preventing contamination would be to prevent the initial attachment of bacteria. In order to determine mechanisms behind bacterial attachment to produce surfaces, the surface structure and biochemical characteristics of both the bacteria and the substratum must be understood. The surface of lettuce and other produce items is covered by a hydrophobic cuticle. It is suspected that hydrophobic bacteria would preferentially attach to those surfaces. One study found that *E. coli* preferentially attached to the hydrophobic waxy cuticle covering the surface of apples (Burnett et al., 2000). It has been suggested that the surface topography, abrasions or the cuticle could reduce the efficacy of sanitizers (Beuchat et al., 1997). It is likely that these factors may also increase adhesion of bacteria.

One of the most studied areas regarding microbial based attachment is dealing with plant pathogens to plant surfaces. The adhesion factors of plant pathogens to plant surfaces could mimic adhesion of foodborne pathogens to the surface of plant foods (Ukuku et al., 2002). Plants and bacteria have a negative surface potential, which may results in electrostatic repulsion between the two surfaces (Ukuku et al., 2002). Surface appendages help the bacteria to overcome this repulsion allowing for specific attachment to the plant surfaces (Ukuku et al., 2002, Romantschuck, 1992). Bacterial attachment is

also thought to be influenced by non-specific factors such as cell surface charge and hydrophobicity (Ukuku et al., 2002, Van der Mei et al., 1991; Van Loosdrecht et al., 1987a; 1987b). The ability to distinguish between specific and non-specific attachment to food surfaces could greatly aid in attachment understanding.

A study by Romantschuk et al. (1993) examined the differences in attachment of piliated and non-piliated *Pseudomonas syringae* to leaf surfaces. They found that the piliated organisms showed preferential adsorption over the non-piliated mutants, thus concluding that surface pili were a significant attachment factor (Romantschuk et al., 1993). Romantschuk also thought that hydrophobic areas on the surface of the plant and hydrophobic areas on the bacterial cell may aid in the initial close contact allowing reversible attachment to occur (1992). While there is little characterization of receptors on plant surfaces, it is thought that fimbriae, specifically, may function as lectins binding to the carbohydrate moieties of plant walls (Romantschuk, 1992). Specific interactions have been seen with type-1 fimbriae and animal tissues, used as a model for plant-microb interactions (Romantschuk, 1992).

SPECIFIC ADHESIVE CELLULAR STRUCTURES OF *ESCHERICHIA COLI*

Bacteria are characterized by their cell wall, as either being Gram positive or Gram negative. Gram positive bacterial cell walls consist mainly of peptidoglycan (a rigid layer creating strength for the cell) with some additional polymers consisting of teichoic acid and/or lipoteichoic acid. Gram negative bacteria have a much more complex cell wall (Madigan and Martinko, 2006). They have a much smaller peptidoglycan layer which is surrounded by an outer membrane. The outer membrane is a second lipid bilayer constructed of phosphlipid, protein, and polysaccharide. The polysaccharide is

attached to the phospholipids creating a lipopolysaccharide layer (LPS). The LPS protrudes from the surface of the cell and contains the cells O-polysaccharide, or O-Somatic antigen which is used to differentiate between *E. coli* species (Madigan and Martinko, 2006).

Bacteria can produce several surface structures which protrude from the cell surface. Some of these include: fimbriae and pili; and capsules and slime layers. *Escherichia coli* O157:H7 has been observed to produce some fimbriae type structures (curli, long polar fimbriae) as well as form exopolysaccharide capsules (Ryu and Beuchat, 2005). These structures may play a role in biofilm formation, and may affect the cells adhesion properties. It has been documented that curli, lps (O-antigen, and intimin all play significant roles in attachment of *E. coli* O157:H7 to intestinal cells (Bilge et al., 1996; Cockerill et al., 1996; McKee and O'Brien, 1996; Sukupolvi et al., 1997; Uhlich et al., 2001; Cornick et al., 2002; Torres and Kaper, 2003; Kim and Kim, 2004). These structures may aid in the attachment of *E. coli* O157:H7 to lettuce surfaces.

A) <u>Curli</u>

Curli structures present on the surface of *E. coli* O157:H7 may affect the cells adhesion to produce surfaces. Curli are very thin, coiled extracellular structures expressed on the surface of most *E. coli* and *Salmonella enteria* strains which bind fibronectin and other proteins (Olsen et al., 1989; Collinson et al., 1996; Romling et al., 1998). These differ from type-1 fimbriae because they are very thin, coiled fibers made up of CsgA monomers (Low et al., 1996). These structures are present on the surfaces of most *E. coli* strains (Brown et al., 2001). In previous studies they have been referred to

as aggregative fimbriae, GVVPQ, or SEF17 fimbriae (Brown et al., 2001; Collinson et al., 2001).

The presence of curli on EHEC surfaces is different for each strain. Several studies have shown a lack of curli production associated with *E. coli* O157:H7 strains (Uhlich et al., 2001; Cookson et al 2002). Cookson et al. (2002) stated that under all growth conditions (25 or 37°C for 24 or 48 hours), there were no appendages present on O157:H7 strain surfaces. Additionally, a survey of curli production in 41 bovine isolates and 8 human strains of *E. coli* O157:H7 proved that only 2 (ATCC 43894 and ATCC 43895) showed very low to moderate curli production (Uhlich et al., 2001). The genes encoding for curli in *E. coli* spp. are the *csg* genes (Brown et al., 2001). These are a conserved cluster of genes regulated by environmental conditions (Brown et al., 2001). The *csgD* gene is thought to be activated by a Crl regulatory protein thought to be activated in response to an environmental stimulant (Arnqvist et al., 1992). Most *E. coli* O157:H7 strains are reported to have a point mutation in the *csgD* promoter region, which is thought to be responsible for the cells lack of curli production (Uhlich et al., 2001; 2002). However, several *E. coli* O157:H7 strains are able to produce curli (ATCC 43894, 43895); these strains do not contain the same mutation as other strains examined (Uhlich et al., 2001; 2002). Brombacher et al. (2000) pointed out that under controlled laboratory conditions, production of curli could be unnecessary for the cell. Recently, Torres and Kaper (2003) discovered curli production in the *E. coli* O157 86-24 wild type strain. They found that when a mutation in the *csgD* gene of the wild type strain was created, the bacteria were no longer to produce curli (Torres and Kaper, 2003).

The expression of curli is dependant of several environmental factors. Curli are also known to assist in biofilm formation; and binding to various cellular structures including: major histocompatability complex class 1 molecules (MHC), contact phase proteins, and soluble extracellular matrix proteins (Brown et al., 2001; Brombacher et al., 2000; Olsen et al., 1989). Biofilm formation increases the survival of bacteria exposed to harsh environmental conditions. As the cells become stressed, they may express curli as a survival factor, allowing cells to aggregate together, and causing cells to adhere to surfaces.

Olsen et al. (1989) identified the temperature dependency of curli expression. When *E. coli* HB101 (which lacks the *crl* gene) was transformed with a plasmid carrying the crl gene (pCRL20), the bacteria were able to express curli and bind fibronectin at 26°C, but not at 37°C (Olsen et al., 1989). Curli expression in the avian pathogenic *E. coli* O78:K80:H9 is also temperature dependant. This strain produced curli when grown at 26 and 37°C, but not at 42°C (Provence and Curtiss, 1992). With the lack of an assay to quantify curli production, their presence was determined through electron microscopy, it was stated that there appeared to be greater production at 26°C than at 37°C (Provence and Curtiss, 1992). The strains ability to bind fibronectin was also determined. The percentage of fibronectin bound was calculated. The fibronectin binding ability of the cells decreased as the temperature increased from 26°C to 37°C, with no fibronectin binding ability demonstrated at the highest temperature (Provence and Curtiss, 1992). The results for this pathogenic strain of *E. coli* are similar to the results obtained previously regarding the HB101 (pCRL20) strain from the Olsen et al. (1989) and Arnqvist et al (1992) studies.

A more recent study analyzing the temperature-regulation of curli production in the avian pathogenic strain was preformed by Maurer et al. (1998). Results from this study were similar to previous described results. Researchers monitored the presence of both *crl* and *csgA* by producing PCR primers for each gene and probing several *E. coli* isolates (using PCR and Southern blot analysis) for their presence (Maurer et al., 1998). They found that *crl* and *csgA* was present in every isolate evaluated, indicating the presence of these genes in a wide variety of *E. coli* strains (Maurer et al., 1998). Researchers then evaluated the expression of curli by growing the cultures in CFA broth at room temperature and at 37°C. Cells were examined by transmission electron microscopy and scanning electron microscopy (Maurer et al., 1998). Cells grown at 37°C only exhibited flagella on their surface, but cells grown at room temperature also exhibited curli production (Maurer et al., 1998). This research further supports the temperature dependence of curli expression in *E. coli* isolates.

Provence and Curtiss (1992) also examined the effect of increasing the osmolarity of the growth media. They found that both the *E. coli* O78:K80:H9 and the HB101 (pCRL20) fibronectin binding ability decreased when CFA agar was supplemented with 0.15 M NaCl (Provence and Curtiss, 1992). Similar results were seen when cultures were grown anaerobically indicating that environmental conditions greatly influenced the expression of curli production and fibronectin binding of the bacteria, leading the researchers to believe that the phenotypes were connected (Provence and Curtiss, 1992). Curli proficient strains were able to produce curli when grown on CFA agar, but not when grown on Luria agar (Olsen et al.,1993). In comparison, CFA has a lower osmolarity than Luria agar (0.15M NaCl). They also added 0.15 M NaCl to CFA plates

to find that curli production has been ceased. Olsen et al. (1993) furthered this experiment by performing Northern blot hybridization with a *csgA* probe. They found that the addition of NaCl decreases the 1150 and 650 base *csgA* transcripts (Olsen et al., 1993). This further supports the osmoregulation of curli expression. A transcription activator of the *csgAB* complex is encoded by the *csgD*, resulting in the production of curli (Brombacher et al., 2003). The expression of *csgD* is regulated by OmpR and EnvZ, a two component regulatory system which monitors osmolarity concentrations in the environment. The creation of a mutation in the *ompR234* gene, allowed for *csgD* to be expressed leading to the production of curli (Brombacher et al., 2000).

The cell's transition into stationary phase growth also increases curli production. Arnqvist et al. (1992) grew a curli proficient strain in colonization factor antigen broth at 26°C. They measured the concentration of *csgA* transcripts over time. They found that during exponential growth, *csgA* was not being transcribed. However, when the cells shifted into stationary phase (24-48hrs), there was marked increase in transcription of *csgA* (Arnqvist et al., 1992). It was suggested that the *crl*, which is enhanced by stationary phase growth, is the trigger for expression of *csgA* (Arnqvist et al., 1992). Bougdour examined Crl levels at various stages of growth, it was found that the level maximized when cell growth stopped, and gradually decreased finally disappearing after 8hours (Bougdour et al., 2004).

While evidence for environmental regulation of curli is abundant; two researchers report that growth temperature and medium did not affect the overproducing strains 43894, 43895 and 4303 (Uhlich et al., 2001; Kim and Kim, 2004). The common method for curli detection includes growing the cells in media with low osmolarity (Colonizing

factor antigen agar, or YESCA) at lower temperatures (22-26°C) for at least 48 hours to enhance expression and plating the bacteria on media supplemented with congo red dye. Curli positive colonies are able to bind the congo red in the agar and produce red colonies whereas, curli negative colonies are white.

The expression of curli may increase the cells ability to adhere to different surfaces. In a survey of 16 strains, Uhlich et al. (2001) found that two *E. coli* O157:H7 curli producing strains ATCC 43894 and 43895 exhibited increased invasion into HEp-2 cells, and increased virulence (Uhlich et al., 2002). Curli formation by *Salmonella* Typhimurium SR-11 significantly increases the cells ability to bind intestinal cells of mice (Sukupolvi et al., 1997). Other researchers confirmed an increased adherence of *E. coli* O157:H7 which over produced curli (strain 4304) (Kim and Kim, 2004). They observed a "massive adhesion pattern" on the surface of HEp-2 cells which was very different from wild type strains (Kim and Kim, 2004).

Curli have been found to also play a role in adherence of the cells to several inert surfaces. Curli overproducing *E. coli* K-12 found to be involved in primary adhesion to polystyrene and thermonox plastic coverslips as well as the formation of biofilm bundles (Prigent-Combaret et al., 2000). Similarly, production of curli by non-O157 STEC was necessary for adherence and biofilm formation on thermonox and glass coverslips (Cookson et al., 2002). Curli have also been shown to increase adhesion of some *E. coli* O157:H7 strains to polystyrene, stainless steel and glass (Pawar et al, 2004). Pawar et al. (2004) also reported that longer cell-surface contact times correlated with greater attachment populations. Cookson et al. (2002) demonstrated that the presence of

fimbriae or curli was associated with attachment of Shiga-toxigenic *E. coli* to abiotic surfaces.

Ryu et al. (2004) found that there were no significant differences between the attachment of E. coli O157:H7 curli proficient and curli deficient strains to stainless steel coupons, following exposure for up to 6 days. Researchers suggested that the hydrophilic nature of stainless steel may hinder attachment, whereas, polystyrene is predominantly hydrophobic, which may explain why other researchers have seen adhesion patterns to abiotic surfaces. The population of curli deficient strains remained fairly constant over the 6 days, with no growth exceeding original populations, however, curli proficient strains did exhibit growth from 5.1 -5.6 $\log_{10}$ CFU/coupon to 7.3-7.9 $\log_{10}$ CFU/coupon within 2 days (Ryu et al, 2004). Researchers found that this was due to biofilm formation between cells; therefore, production of curli significantly influences the cells ability to form biofilms, which may significantly aid the cells survival. Ryu and Beuchat (2005) went on to report that when cells exhibiting curli and extracellular polysaccharide (EPS) were able to form biofilms on stainless steel, they had greater resistance to chlorine.

Research is lacking regarding the relationship between curli production and adhesion to food surfaces. There is significant variation in adhesion of curli producing strains to different food processing surfaces, it is important to explore their relationship with food surfaces. Since curli production seems to be related to adverse environmental stimuli (temperature), cells which come in contact with produce surfaces in the field or processing plants may be able to produce curli and adhere preferentially to surfaces.

B) Lipopolysaccharides:

The lipopolysaccharide (LPS) surface of Gram negative bacteria consists of three parts: Lipid A, the core oligosaccharide, and the O-polysaccharide or O-antigen (Hull, 1997; Heinrichs et al., 1998). The core oligosaccharide links the lipid A to the O-polysaccharide (antigen). Only minimal amounts of lipopolysaccharide are essential for cell viability.

Lipid A is the most important surface structure playing a vital role in replacing phospholipids within the membrane (Hull, 1997). Lipid A is imbedded in the outer membrane. The lipid A component of LPS contains hydrophobic portions of the cell surface, and the O-antigen is predominantly hydrophilic (Hull, 1997). The O-antigen is important because it can move, extending across the cellular surface, covering other molecules in the outer membrane (Hull, 1997). The presence / absence of these different hydrophobic and hydrophilic regions of the outer membrane may be important in attachment of the cells to various surfaces.

The core polysaccharide is highly conserved between bacterial species. This structure extends from Lipid A in the outer membrane and consists of ketodeoxyoctonate (KDO) followed by several six carbon sugars (glucose, galactose) and N-acetylglucosamine (Madigan and Martinko, 2006). There are several variations of this make-up and an abundance of genes that encode for different steps along the way.

O-antigens vary greatly between species. *E. coli* has at least 170 different O-antigens, each signifying a different serotype (Hull, 1997). The O-antigen of *E. coli* O157:H7 is coded by a 14kb gene cluster consisting of 12 genes and an H-repeat unit (Wang and Reeves, 1998). The structure for the O157:H7 O antigens is (in this order):[*N*-acetyl-D-perosamine, L-fucose, D-glucose, and *N*-acetyl-D-galactose] (Wang

and Reeves, 1998). Bacterial cells with an intact O-antigen have smooth colony morphology, whereas cells lacking in O-antigen from rough colonies (Raetz, C., 1996). A very limited number of *E. coli* O-serotypes cause foodborne illness. Diarrheagenic *E. coli* strains are grouped into 6 different vero-types which are defined by the illness that they cause. The viro-type of most concern in the United States is the Enterohemorrhagic (EHEC) strains. The main EHEC serotypes are O26, O111, and O157 (Hull, 1997). The O-antigen is the portion of the cell which is responsible for the immune response mounted by the host (Wang and Reeves, 1998).

A study by Bilge et al. (1996), found that inserting a mutation, Tn*pho*A, randomly throughout the *E. coli* O157:H7 86-24 genome, created a single mutant unable to produce O-antigen. Upon lipopolysaccharide analysis there was no detectable side chain, but the core portion was still intact (Bilge et al., 1996). This mutation was found in an open reading frame, and designated *rfb*$E_{EcO157:H7}$ by researchers, due to its similarity to the *rfbE* gene in *Vibrio cholerae* O1 which encodes perosamine synthetase (Bilge et al., 1996; Stroeher et al. 1986). This LPS negative mutant was found to be 7 times more adherent to epithelial cells than its wild-type parent strain (Bilge et al., 1996). Introduction of plasmid containing *rfb*$E_{EcO157:H7}$ partially restored expression of the O-side chain (Bilge et al., 1996). Authors suggested that a variation in the cell expression of LPS in vivo may occur influencing the cells adhesion to the epithelium (Bilge et al., 1996). Due to the specificity of this gene to *E. coli* O157:H7 it is currently used in multiplex PCR (along with *stx1*, *stx2* and *eaeA*) to rapidly detect bacteria in feces and food products (Nguyen et al, 2004; Wang et al.; 2002, Hu et al., 1999).

Additional research has also examined the role of LPS in adhesion of *E. coli* O157:H7 to various cell lines. With transposon mutagenesis, Cockerill et al. (1996) identified two mutants unable to express the O polysaccharide side chain. These two mutants exhibited greater ability to adhere to HEp-2 cells (Cockerill et al, 1996). Torres and Kaper (2003) confirmed hyper-adherent characteristics of the cell in the absence of the *waaI* gene. The *waaI* gene has been reported to be a galactosyltransferase, responsible for the formation of the LPS core in *Salmonella enterica* serovars (Heinrichs et al., 1998). Previously, Sherman et al. (1988, 1991) provided evidence that outer-membrane constituents do affect the adhesion of *E. coli* O157:H7 to HEp-2 cells.

E. coli O157:H7 has been shown to spontaneously loose its O-antigen under starvation conditions (Hara-Kudo et al., 2000). This loss of antigen may occur under environmental conditions prior to contact of the bacteria with the produce surfaces. Hara-Kudo et al. (2000) found that when surviving under conditions of minimal nutrients (distilled water, and phosphate-buffered saline) 6 of the 19 cultures were negative for O-antigen after 230 days at 18°C, and 1 was negative after 230 days at 4°C. Additionally, Wang and Doyle (1998) found that changes take place in *E. coli* O157:H7 outer membranes after surviving in water. This may be important in the cells adhesion to produce surfaces since it is thought that contamination may occur through contact with untreated water. Environmental conditions in the field or processing plants may also affect the make up of the cells LPS. The presence or absence of some of these components may play a role in attachment of the cells to food surfaces.

C) <u>Attaching and Effacing Mechanism:</u>

The most characterized adhesion factors for *E. coli* O157:H7 is the attaching and effacing mechanism. This allows the bacteria to attach to epithelial cells and produce lesions in the intestine (Meng et al., 2001). A pathogenicity island within the *E. coli* O157:H7 genome, called the locus of enterocyte effacement (LEE), contains the genes involved in the process (Meng et al., 2001; DeVinney et al., 2001; Elliot et al., 1998). A pathogenicity island is a large block of DNA that codes for several virulence factors in pathogenic members of the species, but is absent in non-pathogenic members (Meng et al., 2001). The LEE is 43 kb in size and made up of three segment genes (Meng et al., 2001). These segments include: the *esc* and *sep* genes coding for the type III secretion system; the *eae* and *tir* genes coding for attachment and effacement; and the *esp* genes which induce signal transduction on the epithelial cells (Meng et al., 2001). Tatsuno et al.(2002) showed that mutations in the LEE gene section (*sepL*, *pas*, *sepQ*, *escV*, *sepZ*, *L0038*, *escC*, *escR*, or *L0051)* of the *E. coli* O157:H7 genome decrease attachment by almost 100 percent (Tatsuno et al., 2002). Other researchers, creating specific deletions in *espA*, *eae*, and *sepL* demonstrated the requirement for the presence of these genes in *E. coli* O157:H7 in order for full attachment to occur (Donnenberg et al., 1993; Ebel et al., 1998; Kresse et al., 2000; Nagano et al., 2003).

The attaching and effacing mechanism present in several *E. coli* spp. is a virulence factor promoting colonization of the intestine. Both Enteropathogenic *E. coli* (EPEC) and EHEC show these mechanisms, however they are different from each other. The mechanism found in EPEC strains affects the small intestine, and the mechanisms found in EHEC strains affect the large intestine (Meng et al., 2001). The genetic sequences of the EPEC and EHEC classifications show an overall homology of 83-86%

respectively (Willshaw et al., 1997). This end is thought to be crucial in facilitating the binding of the bacteria the surface of eukaryotic cells (Willshaw et al., 1997).

When EHEC strains are introduced to the intestines, dramatic alterations of the infrastructure of the host mucosal cells occur. This phenomenon is known as "attaching and effacing." Adherence is mediated by a 97-KDa outer membrane protein called intimin, which is coded for by the *eae* genes, and expressed on the outer membrane of the cell (Meng et al.,2001). This initial attachment leads to the effacement of the microvilli surrounding the cell (Meng et al., 2001) Following intimin adherence to the host cell, the bacteria inserts a 78 KDa protein (Tir, translocated intimin receptor) into the host cell via the type III secretion system (DeVinney et al., 2001). The EspA protein forms a needle-like structure which allows bacteria to penetrate into the host cell and secrete Tir and other Esp proteins (Nagano et al., 2003). Once Tir has been inserted, the Tir (within the host cell) and the intimin (on surface of host cell) bind via hydrophobic interactions (DeVinney et al., 2001). As a result of this binding, the actin within the cell is activated and results in a pedestal-like formation on the outside of the cell, which is the intestinal lesion (Meng et al., 2001).

The intimin gene that encodes for this tight "intimate" binding phenomenon is called an "attaching and effacing" gene denoted *eae*A (Salyers et al., 2002). Mutants that lack the *eae* gene have been found to be less virulent indicating that this gene is important in attachment and virulence (Salyers et al., 2002). Insertions in the *eae* gene result in the lack of intimate adherence, however, signal transduction can still be induced (Meng et al., 2001) Studies have shown that *eae* mutants also do not produce the attaching and effacing lesions in some mammals (McKee and O'Brien, 1996; Meng et al., 2001;

Cornick et al., 2002). McKee and O'Brien (1996) found that intimin produced by the *eaeA* gene is essential for intimate adherence and the attaching and effacing to cells in the gnotobiotic pig. Additionally, mutations in the *espA* and *sepL* genes have also been shown to decrease the fecal shedding time in mice inoculated with *E. coli* O157:H7, indicating that these mutants were less able to establish themselves in the mouse intestine (Nagano et al., 2003).

Since these processes play such a role in attachment to epithelial cells in the host, similar reactions may take place on other surfaces. Intimin and Tir bind across the cell membrane via hydrophobic interactions, the presence of intimin expression on the surface of the cell may cause the cell to become more hydrophobic. This hydrophobicity could be key in initializing attachment of the cell to other hydrophobic surfaces.

NON-SPECIFIC ATTACHMENT:

Another influence in bacterial cell's attachment to surfaces may involve less specific factors. The Gram negative cell surfaces are heterogeneous; their physicochemical properties are primarily determined by extracellular polysaccharide appendages (fimbriae). These properties may determine the cells' surface characteristics such as hydrophobicity and cell surface charge, which have been shown to play a role in adhesion of bacteria to various surfaces. The definition of a cells' hydrophobicity is its tendency to associate with similar cells or molecules to in order avoid water (van der Mei et al., 1991), and the surface charge is the net charge carried by the cell (van Loosdrecht et al., 1987b). Polysaccharides and fimbrial appendages previously discussed in this chapter may affect a cell's physicochemical properties. There is potential that correlation between expression of an extracellular structure, their affect on cell properties, and

attachment to surfaces may occur. Seo and Frank (1999) discuss that extra-cellular polymers on the surface of the cell may be hydrophobic, aiding in the mechanism for attachment. *Escherichia coli* and *Salmonella* strains which lack components of the LPS outer surface (rough chemotype), have been shown to have an increased hydrophobicity, relative to a wild type strain (smooth chemotype) (Rosenberg et al., 1980; Fett, 1985). *Salmonella* strains also have a significantly higher cell surface charge when exhibiting a rough LPS chemotype, than those with a smooth chemotype (Fett, 1985). Cookson et al.(2002) did not find an increased hydrophobicity of *E. coli* STEC strains to be related to increased expression of type 1 fimbriae or curli.

Several methods have been examined for effectiveness in determining the physicochemical properties of bacterial cells. To determine cell hydrophobicity, hydrophobic interaction chromatography (HIC), and bacterial adhesion to hydrocarbons (BATH), have been the most widely used procedures (Rosenberg et al., 1980; Fett, 1985; Mozes and Rouxhet, 1987; Dickson and Koohmaraie, 1989; Benito et al., 1997; Li et al., 1999, Cookson et al., 2002; Ukuku and Fett, 2002; Rivas et al., 2005) Common methods for determining cell charge include electrostatic interaction chromatography (ESIC) and measurement of zeta potential to determine electrophoretic mobility (Pedersen, 1980; van Loosdrecht et al., 1987; Dickson and Koohmaraie, 1989; Harkes et al., 1992; Lytle et al., 1999; Braindet et al., 1999a; Li et al., 1999; Ukuku and Fett, 2002). It can be difficult to compare articles due to the abundance of methods used, however it has been found that results obtains using such methods typically agree (Mozes and Rouxhet, 1987).

A) <u>Hydrophobicity and Cell Charge:</u>

Hydrophobic interaction chromatography (HIC) is completed by passing bacteria through a column packed with hydrophobic gel, most commonly, octyl sepharose or phenyl sepharose. The initial washed bacterial cell concentration of inoculum is determined. Following elution through the column, the eluate is analyzed for bacteria concentration. Eluate concentration (*e*) is subtracted from inoculum concentration to give the number of cells retained in the column (*g*). Then the hydrophobicity value is expressed as either the ratio *g/e*, or as percentage retained in the column (% hydrophobic). This method was first described by Dahlback et al. (1981). The BATH method is very similar. Initial washed bacterial cell concentration is determined, and then mixed in the presence of liquid hydrocarbon. The phases are then allowed to separate. Cells which adhere to the hydrocarbon will rise to the top of the solution forming an upper layer. The number of cells attached is determined by analyzing the lower aqueous phase. Results are expressed as ratio of bacterial aqueous layer to the initial bacterial cell suspension. Results are typically expressed at a percentage. HIC is typically used to demonstrate differenced between cells that are more hydrophilic in nature, while the BATH method is better suited to differentiate between cells which are highly hydrophobic (Rosenberg et al., 1980; Fett, 1985).

Electrostatic interaction chromatography, a method similar to HIC, has been used to determine a cell's overall charge. This method uses the same technique but passes the bacteria through a column packed with either anion exchange or cation exchange resin (chloride, or hydrogen form). Data has been presented either as *r/e*, where *r* is number retained in the column and *e* is number eluted from column, or as a percentage of cells

retained in the column. The other method evaluates the measure of the magnitude of the repulsion or attraction between particles, this is called the zeta potential.

Wild type *E. coli* O157:H7 has been found to have a relatively low surface hydrophobicity with data ranging between 0.202 and 0.233 when analyzed using the HIC method (Sherman et al., 1987; Dickson and Koohmaraie, 1989; and Ukuku and Fett, 2002) and < 25% when using the BATH test (Rosenberg et al., 1980; Dewanti and Wong, 1995; Li and McLandsborough, 1999; Cookson et al., 2002; Hassan and Frank, 2004). Hydrophobicity varies between strains and serotypes of bacteria. Several strains of *E. coli* O157 and O128:H2 varied between 1.7 and 14.33% hydrophobicity. Other researchers found similar results where the variation in HIC values (expressed as a percentage) between serotypes ranged from 2.9 to 33.4% (Rivas et al., 2005).

In general, *E. coli* O157:H7 has an overall negative charge. However, the O157:H7 serotype exhibits a less negative cell charge than other shiga-toxigenic *E .coli* (STEC) (Lytle et al., 1999; Rivas et al., 2005). When analyzing the physicochemical properties of *E. coli* O157:H7, Rivas et al. (2005), found that the zeta potential of 7 *E. coli* O157:H7 (ranging from-8.0mV to 1.0mV), in a group of 20 strains had a cell charge that was significantly less negative than several non-O157 serotypes (-15 to -38mV). These results are consistent with Lytle et al. (1999). They found that the 12 O157:H7 isolates examined had significantly less negative cell charge from the 5 wild type analyzed (Lytle et al., 1999). Other researchers have also reported a relatively low negative cell charge of *E. coli* O157:H7 using the zeta potential as a measurement (between -3.34 and -7.68mV) (Hassan and Frank, 2004). Results are consistent using the ESIC methods, Dickson and Koohmaraie (1989) reported an overall negative cell surface

charge for *E. coli* when they performed ESIC method. A net negative charge of 1.33 and a net positive charge of 0.16 was reported (Dickson and Koohmaraie, 1989). Several other bacteria examined had much higher negative charges. In comparison, *Listeria monocytogenes, Staphyoccocus aureus, and Salmonella* Typhimurium had much greater overall negative charge (Dickson and Koohmaraie, 1989). Ukuku and Fett (2002) found similar results when using EISC as a method to measure cell charge. When they surveyed two O157:H7 strains and the ATCC strain 25922, they found that there were more negative surface charges (1.48-1.62) than positive surface charges (0.12-0.18) (Ukuku and Fett, 2002). Other research has also reported that *E. coli* spp. have an overall negative surface charge (van Loosdrecht et al., 1987b)

Variations in *L. monocytogenes* subculture, growth conditions and storage have been shown to significantly alter a cell's hydrophobicity (Briandet et al.1999a,b). When assaying two processing plant strains (*L.monocytogenes* D, M) and one human isolate (Scott A), it was found that the subtypes varied significantly in their hydrophobicity (Briandet et al., 1999b). Storage of *L monocytogenes* at 4°C and -80°C alters the cells hydrophobicity; *L.monocytogenes* M was more hydrophobic when stored at 4°C instead of -80°C (Briandet et al., 1999a,b). Consistency of the cells hydrophobic characteristic was increased when grown at -80°C than when grown at 4°C (Briandet et al., 1999a,b). Researchers also determined that the fewer the transfers, the less variable the data. They concluded that for hydrophobicity or adhesion assays it was beneficial to store cultures at lower temperatures and minimize growth transfers during the experiments (Briandet et al., 1999a,b). Cells have also exhibit higher hydrophobicity at high growth rates, and

when grown in broth versus agar medium (van Loosdrecht et al., 1987b; Rivas et al., 2005).

Growth media has also been shown to affect the cells hydrophobicity. Cells grown in Tryptic soy broth were significantly less hydrophobic that those grown in nutrient broth (Hassan and Frank, 2004). Dewanti and Wong (1995) found that when *E. coli* O157:H7 was grown in minimal salt medium, the cells were more hydrophobic (5.3-8.7%, BATH) than those grown in TSB (0%). It has been hypothesized that this may be why they attach preferentially to hydrophobic apple surfaces, including the waxy cuticle, lenticels, and russet areas (Burnett et al., 2000; Seo and Frank, 1999).

The role of bacterial hydrophobicity in adhesion to a variety of surfaces in not clearly defined. Greater adherence ability to inert surfaces has been reported. When analyzing several bacterial strains, Van Loosdrecht (1987a) found that hydrophobic cells have a better adherence to sulfated polystyrene than hydrophilic cells. They state in a separate paper that bacterial cells tend to be more hydrophibic at higher growth rates, and that greater adhesion is also correlated with higher growth rates (van Loosdrecht 1987b). Surface hydrophobicity also influenced attachment of *L. monocytogenes* to stainless steel surfaces. Greater attachment to stainless steel correlated with greater hydrophobicity (Briandet et al., 1999b). One study reports preferential attachment of *E. coli* O157:H7 to rubber and polystyrene (hydrophobic surfaces) than stainless steel and glass (hydrophilic) in that order (Pawar et al., 2004). This study shows that cells preferentially attached to the hydrophobic surface over the hydrophilic. Research to better understand the adhesion of plant pathogens to plant surfaces indicates that bacterial strains exhibiting lower surface hydrophobicity were typically less likely to become immobilized on soybean

leaves therefore concluding that increased surface hydrophobicity was an important factor in adhesion (Fett, 1985).

Pathogenic and spoilage bacteria associated with meat were examined by Benito et al (1997). Researchers labeled cells which were easily removed in rinse water prior to adhesion experiment "loosely" attached and those which were only enumerated by homogenization of the meat "tightly" attached. With these methods, it was demonstrated that hydrophobicity does not correlate with cell attachment, but with attachment strength of the cells (tightly attached) (Benito et al, 1997). Dickson and Koohmaraie (1989) reported a significant correlation between hydrophobicity and attachment to beef fat.

Ukuku and Fett (2002), using similar methods as Benito et al., also described a linear correlation between surface charge and hydrophobicity in relation to the attachment strength of *Salmonella*, *L. monocytogenes*, and *E. coli* O157:H7 to the surface of cantaloupe rinds. Bacteria remained attached over a period of seven days with the strongest attachment by *Salmonella* spp. and weakest for *Listeria* spp. (Ukuku and Fett, 2002).

In opposition, some researchers have reported little or no correlation between hydrophobicity of the cell and attachment. Hassan and Frank (2004) found that the more hydrophobic the cell, the less likely it was to attach to lettuce and apple surfaces, therefore they concluded that other cell characteristics such as cell charge or the presence of extracellular polysaccharides may be more influential on attachment (Hassan and Frank, 2004). It has also been reported that there is no correlation between hydrophobicity and cell adhesion to beef muscle (Dickson and Koohmaraie, 1989; Li et al., 1999).

Research relating a cell's charge to its adhesion characteristics, is not as convincing. Briandet et al. (1999) found a correlation between the highly negative cell surface charge of *L. monocytogenes* and its attachment to stainless steel surfaces. A linear correlation between the negative surface charge of a bacterial cell and attachment to beef muscle has been reported, where no relation in hydrophobicity was found (Dickson and Koohmaraie, 1989).

No correlation was found between surface charge alone, and the cells ability to attach to stainless steel surface (van Loosdrecht et al., 1987b). Although researchers found that hydrophobicity was the key factor, as cells became less hydrophobic, it is noted that the cell charge begins to influence attachment (van Loosdrecht et al., 1987b). It was noted that cells exhibiting high hydrophobic characteristics tended to also have a large negative charge (van Loosdrecht et al., 1987b). No correlation has been seen between cell surface charge and plant immobilization, or adhesion to beef muscle or apple and lettuce surfaces (Fett, 1985; Li and McLandsborough, 1999; Hassan and Frank, 2004).

REFERENCES

Ackers, M. L., B. E. Mahon, E. Leahy, B. Goode, T. Damrow, P. S. Hayes, W. F. Bibb, D. H. Rice, T. J. Barrett, L. Hutwagner, P. M . Griffin, and L. Slutsker. 1998. An outbreak of *Escherichia coli* O157:H7 infections associated with leaf lettuce consumption. *J. Infect. Dis*. 177:1588-1593.

Ahvenainen, R. 2000. Minimal processing of fresh produce. p. 227-290. *In* S. M..Alzamora, M. S. Tapia, and A. Lopez-Malo (ed.), Minimally processed fruits and vegetables. Aspen Publications. Gaithersburg, MD.

Arnqvist, A., A. Olsen, J. Pfeifer, D.G. Russell, and S. Normark. 1992. The Crl protein activates cryptic genes for curli formation and fibronectin binding in *Escherichia coli* HB101. *Mol. Microbiol*. 6:2443-2452.

Benito, Y., C. Pin, M. Luisa Martin, M. Luisa Garcia, M. Delores Selagas and C. Casas. 1997. Cell Surface hydrophobicity and attachment of pathogenic and spoilage bacteria to meat surfaces. *Meat Science*. 45:419-425.

Beuchat, L. R., and J. H. Ryu. 1997. Produce handling and processing practices. *Emerg Infect Dis*. 3:459-465.

Beuchat, L. R., B. V. Nail, B. B. Adler, and M. R. S. Clavero. 1998. Efficacy of spray application of chlorinated water in killing pathogenic bacteria on raw apples, tomatoes, and lettuce. *J. Food Prot.* 61:1305-1311.

Besser, R. E., S. M. Lett, J. T. Weber, M. P. Doyle, T. J. Barrett, J. G. Wells and P. M. Griffin. 1993. An outbreak of diarrhea and hemolytic uremic syndrome from *Escherichia coli* O157:H7 in fresh-pressed apple cider. *JAMA*. 269:2217-2220.

Bilge, S. S., J. C. Vary, S. F. Dowell, and P. I. Tarr. 1996. Role of *Escherichia coli* O157:H7 O-side chain in adherence and analysis of an *rfb* locus. *Infect. Immun.*. 64:4795-4801.

Borriss, H., and H. Brunke. 2005. Commodity Profile: Lettuce. Agricultural marketing resource center. University of California. [INTERNET, WWW] http://aic.ucdavis.edu/profiles/lettuce-2005.pdf.

Briandet, R., T. Meylheuc, C. Maher, and M. N. Bellon-Fontaine. 1999a. *Listeria monocytogenes* Scott A: cell surface charge, hydrophobicity, and electron donor and acceptor characteristics under different environmental growth conditions. *Appl. Environ. Microbiol*. 65:5328-5333.

Briandet, R., V. Leriche, B. Carpentier, and M. N. Bellon-Fontaine. 1999b. Effects of the growth procedure on the surface hydrophobicity of *Listeria monocytogenes* cells and their adhesion to stainless steel. *J. Food Prot*. 62:994-998.

Brombacher, E., C. Dorel, A. J. B. Zehnder, and P. Landini. 2003. The curli biosynthesis regulator CsgD co-ordinates the expression of both positive and negative determinants for biofilm formation in *Escherichia coli*. *Microbiol*. 149:2847-2857.

Brooks, G. F., J. S. Butel, and S. A. Morse. 1998. *Medical Microbiology*. Appleton & Lange, Stamford, Conn.

Brown, P. K., C. M. Dozois, C. A. Nickerson, A. Zuppardo, J. Terlonge, and R. Curtiss, IIII. 2001. MirA, a novel regulator of curli (AgF) and extracellular matrix synthesis by *Escherichia coli* and *Salmonella enterica* serovars Typhimurium. *Mol. Microbiol*. 41:349-363.

Burnett, S. L., J. Chen, and L. R. Beuchat. 2000. Attachment of *Escherichia coli* O157:H7 to surfaces and internal structures of apples as detected by confocal scanning laser microscopy. *Appl. Environ. Microbiol*. 66:4679-4687.

Cabedo, L., J. N. Sofos, G. R. Schmidt, and G. C. Smith. 1997. Attachment of *Escherichia coli* O157:H7 and other bacterial cells grown in two media to beef adipose and muscle tissues. *J. Food Prot*. 60(2):102-106.

Center for Disease Control and Prevention. 1996. Outbreaks of *Escherichia coli* O157:H7 infections associated with drinking un-pasteurized commercial apple juice—British Columbia, California, Colorado, and Washington, Oct 1996. *MMWR*. 45:975.

Center for Disease Control and Prevention. 1997b. Outbreaks of *Escherichia coli* O157:H7 infection and cryptosporidiosis associated with drinking un-pasteurized apple cider--Connecticut and New York, October 1996. *MMWR*. 46:4-8.

Center for Disease Control and Prevention. 2002a. Press Release, Update: *E. coli* O157:H7 Ground Beef Investigation. Office of communications. [Internet, WWW] ADDRESS: http://www.cdc.gov/od/oc/media/pressrel/b020813.htm.

Center for Disease Control and Prevention. 2002b. Multistate outbreak of *E. coli* O157:H7 Infections associated with eating ground beef—United States June-July 2002. *MMWR*. 51(29)637-639.

Center for Disease Control and Prevention. 2002c. U.S. Foodborne disease outbreaks. [Internet, WWW] ADDRESS: http://www.cdc.gov/ncidod/dbmd/outbreak/us_outb.htm.

Center for Food Safety and Nutrition (CFSAN). 2004. Letter to Firms that Grow, Pack, or Ship
Fresh Lettuce and Fresh Tomatoes. [Internet, WWW] ADDRESS: http://www.cfsan.fda.gov/~dms/prodltr.htm

Cockerill, F., III, F. Beebakhee, R. Soni, and P. Sherman. 1996. Polysaccharide side chains are not required for attaching and effacing adhesion of *Escherichia coli* O157:H7. *Infect. Immun*. 64:3196-3200.

Cookson, A. L., W. A. Cooley, and M. J. Woodward. 2002. The role of type 1 and curli fimbriae of shiga toxin-producing *Escherichia coli* in adherence to abiotic surfaces. *Int. J. Med. Microbiol*. 292: 195-205.

Collinson, S. K., L. Emody, T. J. Trust, and W. W. Kay. 1992. Thin aggregative fimbriae from diarrheagenic *Escherichia coli*. *J. Bacteriol*. 174:4490-4495.

Cornick, N. A., S. L. Booher, and H. W. Moon. 2002. Intimin facilitates colonization by *Escherichia coli* O157:H7 in adult ruminants. *Infect. Immun*. 70(5):2704-2707.

Dahlback, B., M. Hermansson, S. Kjelleberg, and B. Norkrans. 1981. The hydrophobicity of bacteria—an important factor in their initial adhesion at the air-water interface. *Arch. Microbiol*. 128:267-270.

Deshpande, S. S., and D. K. Salunkhe. 1998. Lettuce, 493-509. In D. K. Salunkhe, and S. S. Kadam (ed). Handbook of vegetable science and technology. Marcel Dekker, Inc. New York, NY.

DeVinney, R., J. L. Puente, A. Gauthier, D. Goosney, and B. B. Finlay. 2001. Enterohemorrhagic and enteropathogenic *Escherichia coli* use a different Tir-based mechanisms for pedestal formation. *Molecular Microbiology*. 41(6):1445-1458.

Dewanti, R., and A.C.L. Wong. 1995. Influence of culture conditions on biofilm formation by *Escherichia coli* O157:H7. *Int. J. Food Microbiol*. 26:147-167.

Dickson, J. S., and M. Koohmaraie. 1989. Cell surface charge characteristics and their relationship to bacterial attachment to meat surfaces. *Appl. Environ. Microbiol*. 1989. 55:832-836.

Donnenberg, M. S., S. Tzipori, M. L. McKee, A. D. O'Brien, J. Alroy, and J. B. Kaper. 1993. The role of the eae gene of Enterohemorrhagic *Escherichia coli* in intimate attachment in vitro and in a porcine model. *J. Clin. Invest*. 92:1418-1424.

Doyle, M. P., and Cliver, D. O. 1990. *Escherichia coli*, p. 209-215. *In* D.O. Cliver (ed.), *Foodborne Diseases*. Academic Press Inc. New York, NY.

Ebel, R. T., T. Podzadel, M Rohde, A. U. Kresse, S. Kramer, C. Deibel, C. A. Guzman, and T. Chakraborty. 1998. Initial binding of Shiga toxin-producing *Escherichia coli* to

host cells and subsequent induction of actin rearrangement depend on filamentous EspA-containing surface appendages. *Mol. Microbiol*. 30:147-161.

Eckert, J. W. 1980. Post harvest disease of fresh fruits and vegetables – Etiology and control, p. 81-117. *In* N.F. Haard, and D.K. Salunkhe, (ed.), Post harvest biology and handling of fruits and vegetables. The AVI Publishing Company INC.

Elliot, S.J., L. A.,Wainwright, T. K. McDaniel, K. G. Jarvis, Y. K. Deng, L. C. Lai, B. P. MacNamara, M. S. Donnenberg, and J. B. Kaper. 1998. The complete sequence of the locus of enterocyte effacement (LEE) from EPEC E2348/69. *Mol. Microbiol*. 28:1-4.

Farrell, B. L., A. B. Ronner, and A. C. Lee Wong. 1998. Attachment of *Escherichia coli* O157:H7 in ground beef to meet grinders and survival after sanitation with chlorine and peroxyacetic acid. *J. Food Prot.* 61:817-822.

Fett, W. F. 1985. Relationship of bacterial cell surface hydrophobicity and charge to pathogenicity, physiologic race and immobilization in attached soybean leaves. *Physiol. Biochem*. 75:1414-1418.

Food and Drug Administration. 2001. Survey of imported fresh produce. [Internet, WWW], ADDRESS: http://vm.cfsan.fda.gov/~dms/prodsur9.html.

Francis, G. A., C. Thomas, and D. O'Beirne. 1999. The microbiological safety of minimally processed vegetables. *Int. J Food Sci and Tech*. 34:1-22.

Fratamico, P. M., F. J. Schultz, R. C. Bendict, R. L. Buchanan, and P. H. Cooke. 1996. Factors influencing attachment of *Escherichia coli* O157:H7 to beef tissues and removal using selected sanitizing rinses. *J. Food Prot*. 59(5):

Garg, N., J. J. Churey, and D. F. Splittstoesser. 1990. Effect of processing conditions on the microflora of fresh cut vegetables. *J of Food Prot*. 53:701-703.

Greenleaf, C. 1999. Making the cut. Amer. Veg. Grower. March:10-11.

Hara-Kudo, Y., M. Miyahara, and S. Kumagai. 2000. Loss of O157 O antigenicity of verotoxin-producing *Escherichia coli* O157:H7 surviving under starvation conditions. *Appl. Env. Microbiol.* 66(12):5540-5543.

Harkes, G., H C van der Mei, P. G. Rouxhet, J. Dankert, H. J. Busscher, and J. Feijen. 1992. Physicochemical characterization of *Escherichia coli*. A comparison with gram-positive bacteria. *Cell Biophys*. 20:17-32.

Hassan, A. N., and J. F. Frank. 2004. Attachment of *Escherichia coli* O157:H7 grown in tryptic soy broth and nutrient broth to apple and lettuce surfaces as related to cell hydrophobicity, surface charge, and capsule production. *Int. J. Food Microbiol*. 96:103-109

Heinrichs, D. E., J. A. Yethon, and C. Whitfield. 1998. Molecular basis for structural diversity in the core regions of the lipopolysaccharides of *Escherichia coli* and *Salmonella enterica*. *Mol. Micriobiol*. 30:221-232.

Hilborn, E. D., J. H. Mermin, P. A. Mshar, J. L. Hadler, A. Voetsch, C. Wojtkunski, M. Swartz, R. Mshar, M. A. Lambert-Fair, J. A. Farrar, M. K. Glynn, and L. Slutsker. 1999. A multistate outbreak of *Escherichia coli* O157:H7 infections associated with consumption of mesclun lettuce. *Arch. Intern. Med.* 159:1758-1764.

Hitchens, A. D., P. Feng, W.D. Watkins, S. R. Rippey, and L. A. Chandler. 1995. *E. coli* and coliform bacteria. P. 4.01-4.28. *In* FDA bacteriological analytical manual, 8th ed. Association of Official Analytical Chemists, Gaithersburg, MD.

Hodge, K. 1995. Fresh-cut an the "perfect meal," *Fresh Cut*. 3:12.

Hu, Y., Q. Zhang, and JC. Meitzler. 1999. Rapid and sensitive detection of *Escherichia coli* O157:H7 in bovine faeces by multiplex PCR. *J Appl. Microbiol*. 87:867-876.

Hull, S. 1997. *Escherichia coli* lipoploysaccharide in pathogenesis and virulence, p. 145-167. *In* M. Sussman (ed.), *Escherichia coli*: mechanisms of virulence. Cambridge University Press, United Kingdom.

Hurst, W. C., and G. A. Schuler. 1992. Fresh produce processing – and industry perspective. *J. Food Prot*. 55:824-827.

IFPA. International Fresh Cut Produce Association. 2001, http://fresh-cuts.org

Jay, J.M. 2000. *Modern Food Microbiology*. Aspen Publishers, Inc. Gaithersburg, MD. 531-547.

Kaufman, P.R., C. R. Handy, E. W. McLaughlin, K. Park, and G. M. Green. 2000. Understanding the dynamics of produce markets consumption and consolidation growth. USDA ERS. Agricultural Information Bulletin number 758.

Kim, SH., and YH Kim. 2004. *Escherichia coli* O157:H7 adherence to HEp-2 cells is implicated with curli expression and outer membrane integrity. *J. Vet Sci*. 5:119-124.

Kresse, A. U., F. Beltrametti, A. Muller, R. Ebel, and C. A. Guzman. 2000. Characterization of SepL of Enterohemorrhagic *Escherichia coli*. *J. Bacteriol*. 182:6490-6498.

Kudva, I.T., S. Jelacic, P.I. Tarr, P. Youderian, and C. J. Hovde. 1999. Biocontrol of *Escherichia coli* O157:H7 with O157-specific bacteriophages. *Infect. Immun*. 65:3767-3773.

Li, Y. R.E. Brackett, J. Chen, and L.R. Beuchat. 2001. Survival and growth of *Escherichia coli* O157:H7 inoculated onto cut lettuce before or after heating in chlorinated water, followed by storage at 5 or 15°C. *J. Food Prot*. 64: 305-309.

Li, J., L. A. McLandsborough. 1999. The effects of the surface charge and hydrophobicity of *Escherichia coli* on its adhesion to beef muscle. *Int. J. Food Microbiol*. 53:195-193.

Liao, CH., and G. M. Sapers. 2000. Attachment and growth of *Salmonella chester* on apple fruits and in vivo response of attached bacteria to sanitizer treatments. *J. Food Prot.* 63:876-883.

Low, D., B. Braaten, and M. van der Woude. 1996. Fimbriae. *In* Neidhardt, F. C. (ed.), *Escherichia coli* and *Salmonella*, cellular and molecular biology, 2nd ed. ASM Press, Washington, D.C.

Lytle, D. A., E. W. Rice, C. H. Johnson, and K. R. Fox. 1999. Electrophoretic mobilities of *Escherichia coli* O157:H7 and wild-type *Escherichia coli* strains. *Appl. Env. Microbiol*. 65:3222-3225.

Madigan, M.T., and J. M. Martinko (ed.). 2006. Brook Biology of Microorganisms, 11th ed. Peason Prentice Hall. Upper Saddle River, NJ.

Maurer, J. J., T. P. Brown, W. L. Steffens, and S. G. Thayer. 1998. The occurance of ambient temperature-regulated adhesins, curli, and the temperature-sensitive hemagglutinin Tsh among avian *Escherichia coli*. *Avian Diseases*. 42:106-118.

McKee, M. L., and A. D. O'Brien. 1996. Truncated enterohemorrhagic *Escherichia coli* (EHE) O157:H7 intimin (EaeA) fusion proteins promote adherence of EHEC strains to Hep-2 cells. *Infect. Immun.*. 64:2225-2233.

Mead, P. S., L. Slutsker, V. Dietz, L. F. McCaig, J. S. Bresee, C. Shapiro, P. M. Griffin, and R. V. Tauxe. 1999. Food-related illness and death in the United States. *Emerg. Infect. Dis.* 5:607-625.

Meng, J., M. P. Doyle, T. Zhao, and S. Zhao. 2001. Enterohemorrhagic *Escherichia coli*, 193-213. *In* Doyle, M. P., L. R. Beuchat, and T. J. Montville (ed), Food Microbiology: fundamentals and frontiers, 2nd ed. ASM Press, Washington D.C.

Montville, T. J., and K. R. Matthews. 2001. Principles which influence microbial growth, survival, and death in foods, pp. 13-32. *In* Doyle, M. P., L. R. Beuchat, and T. J. Montville (ed), Food Microbiology: fundamentals and frontiers, 2nd ed. ASM Press, Washington D.C.

Mozes, N., and P. G. Rouxhet. 1987. Methods for measuring hydrophobicity on microorganisms. *J. Microbiol. Methods*. 6:99-112.

Nagano K., K. Taguchi, T. Hara, S. Yokoyama, K. Kawada, and H. Mori. 2003. Adhesion and colonization of Enterohemorrhagic *Escherichia coli* O157:H7 in cecum of mice. *Microbiol. Immunol*. 47:125-132.

National Cancer Institute. 2003. Eat 5 to 9 a day for better health, about the program. [Internet,WWW] ADDRESS: www.5aday.gov/index-about.shtml.

Nguyen, L. T., BE. Gillespie, HM. Nam, SE. Murinda, and SP. Oliver. 2004. Detection of *Escherichia coli* O157:H7 and *Listeria monocytogenes* in beef products by real time polymerase chain reaction. *Foodborne pathog. Dis*. 1:231-240.

Olsen, A., A. Jonsson, and S. Normark. 1989. Fibronectin binding mediated by a novel class of surface organelles on *Escherichia coli*. *Nature*. 338, 652-655.

Olsen, A., A. Arnqvist, M. Hammar, S. Sukupolvi, and S. Normark. 1993. The RpoS sigma factor relieves H-NS-mediated transcriptional repression of *csgA*, the subunit gene of fibronectin-binding curli in *Escherichia coli*. *Mol Microbiol*. 7:523-536.

Pawar, D. M., M. L. Rossman, and J. Chen. 2005. Role of curli fimbriae in mediating the cells of enterohaemorrhagic *Escherichia coli* to attach to abiotic surfaces. *J. Appl. Micro*. 99:418-425.

Pederson, K. 1980. Electrostatic interaction chromatography, a method for assaying the relative surface charges of bacteria. *FEMS Microbiol. Lett*. 12:365-367.

Prigent-Combaret, C., G. Prensier, T.T. Le Thi, O. Vidal, P. Lejeune, and C. Dorel. 2000. Developmental pathway for biofilm formation in curli-producing Escherichia coli strains: role of flagella, curli, and colonic acid. *Environ. Microbiol*. 4:450-64.

Provence, D. L., and R. Curtiss III. 1992. Role of *crl* in avian pathogenic *Escherichia coli*: a knockout mutation of *crl* does not affect Hemagglutination activitiy, fibronectin binding or curli production. *Infect. Immun*. 60:4460-4467.

Raetz, C.R.H. 1996. Bacterial lipopolysaccharides: a remarkable family of bioactive macroamphiphilesm, 1035-1063. *In Escherichia coli* and *Salmonella*. Cellular and molecular biology. Neidhardt, F. C., R. Curtiss, J. L. Ingraham, E. C. C. Lin, and K. B. Low (ed). ASM press, Washington D.C.

Rajkowski, K. T., and E. A. Baldwin. 2003. Concerns with minimal processing in apple, citrus, and vegetable products, pp. 35-52. *In* J. Noval, G. M. Sapers, and V. K. Juneja(ed.), Microbial safety of minimally processed foods . CRC Press, Washington, D.C.

Rangel, J. M., P. H. Sparling, C. Crowe, P. M. Griffin, and D. L. Swerdlow. 2005. Epidemiology of *Escherichia coli* O157:H7 outbreaks, United States, 1982-2002. *Emerg. Infect. Dis.* 11:603-609.

Rivas, L., N. Fegan, and G. A. Dykes. 2005. Physicochemical properties of shiga toxigenic *Escherichia coli*. *J. Appl. Microbiol*. 99:716-727

Roberts, T. A, J. I. Pitt, J. Farkas, and F. H. Grau, (ed.). 1998. Microorganisms in foods 6: microbial ecology of food commodities. Blackie Academic & Professional, New York, NY.

Romanschuk, M. 1992. Attachment of plant pathogenic bacteria to plant surfaces. *Ann. Rev. Phytopathol*. 30:225-243.

Romanschuk, M., EL. Nurmiaho-Lassila, E. Roine, A. Suoniemi. 1993. Pilus-mediated adsorption of *Pseudomonas syringae* to the surface of host and non-host plant leaves. *J. Gen. Microbiol*. 139:2251-2260.

Rosenberg, M., D. Gutnick and E. Rosenberg. 1980. Adherence of bacteria to hydrocarbons: a simple method for measuring cell-surface hydrophobicity. *FEMS Microbiol. Lett*. 9:29-33.

Ryu, J. H., H. Kim, J. F. Frank, and L. R. Beuchat. 2004. Attachment and biofilm formation on stainless steel by *Escherichia coli* O157:H7 as affected by curli production. *Lett Appl Microbiol* 39**:**359-362

Ryu, J. H., and L. R. Beuchat. 2005. Biofilm formation by *Escherichia coli* O157:H7 on stainless steel: effect of exopolysaccharide and Curli production on its resistance to chlorine. *Appl Environ Microbiol* 71**:**247-254.

Salyers, A. A., and D. D. Whitt. 2002. Bacterial Pathogenisis: a molecular approach. ASM Press. Washington D.C.

Seo, K. H., and J. F. Frank. 1999. Attachment of *Escherichia coli* O157:H7 to lettuce leaf surface and bacterial viability in response to chlorine treatment as demonstrated by using confocal scanning laser microscopy. *J. Food Prot*. 62:3-9.

Sherman, P., F. Cockerill III, R. Soni, and J. Brunton. 1991. Outer membranes are competitive inhibitors of *Escherichia coli* O157:H7 adherence to epithelial cells. *Infect. and Immun.*. 59: 890-899.

Sherman, P., and R. Soni. 1988. Adherence of vero cytotoxin-producing *Escherichia coli* of serotype O157:H7 to human epithelial cells in tissue culture: role of outer membrane as bacterial adhesins. *J. Med. Microbiol*. 26:11-17.

Sherman, P., R. Soni, M. Petric, and M. Karmali. 1987. Surface properties of vero cytotoxin producing *Escherichia coli* O157:H7. *Infect. Immun.*. 55: 1824-1829.

Sivapalasingam, S., C. R. Freidman, L. Cohen, and R. V. Tauxe. 2004. Fresh produce: a growing cause of outbreaks of foodborne illness in the United States, 1973 through 1997. *J. Food Prot*. 67:2342-2353.

Stroeher, U. H., L. E. Karagerogos, R. Morona, and P. A. Manning. 1992. Serotype conversion in *Vibrio cholerae* O1. *Proc. Natl. Sci*. USA 89:2566-2570.

Sukupolvi, S., R. G Lorenz, J. I. Gordon, Z. Bian, J. D. Pfeifer, S. J. Normark, and M. Rhen. 1997. Expression of thin aggregative fimbriae promotes interaction of *Salmonella* typimurium SR-11 with mouse small intestinal epithelia cells. *Infect Immun.*. 65:5320-5325.

Takeuchi, K., and J. F. Frank. 2001a. Expression of Red-shifted green fluorescent protein by *Escherichia coli* O157:H7 as a marker for detection of cells on fresh produce. *J. Food Prot*. 64:298-304

Takeuchi, K., and Frank, J. F.. 2001c. Quantitative determination of the role of lettuce leaf structures in protecting *Escherichia coli* O157:H7 from chlorine disinfection. *J. Food Prot*. 64:147-151.

Takeuchi, K., A. N. Hassan, and J. F. Frank. 2001. Penetration of *Escherichia coli* O157:H7 into lettuce as influenced by modified atmosphere and temperature. *J. Food Prot*. 64:1820-1823.

Takeuchi, K., C. M. Matute, A. N. Hassan, and J. F. Frank. 2000. Comparison of attachment of *Escherichia coli* O157:H7, *Listeria monocytogenes*, *Salmonella typhimurium*, and *Pseudomonas fluorescens* to lettuce leaves. *J. Food. Prot*. 63:1433-1437.

Tatsuno, I., H. Kimura, A. Okutani, K. Kanamaru, H. Abe, S. Nagai, K. Makino, H. Shinagawa, , M. Yoshida, K. Sato, J. Nakamoto, T. Tobe, and C. Sasakawa. 2000. Isolation and characterization of Mini-Tn5Km2 insertion mutants of Enterohemorrhagic *Escherichia coli* O157:H7 deficient in adherence to Caco-2 cells. *Infect. Immun*. 68(10):5943-5952.

Tauxe, R., H. Kruse, C. Hedberg, M. Potter, J. Madden, and K. Wachsmuth. 1997. Microbiological hazards and emerging issues associated with produce: A preliminary report to the National Advisory Committee on Microbiologic Criteria for Foods. *J. Food Prot*. 60:1400-1408.

Torres, A. G., and J. B. Kaper. 2003. Multiple elements controlling adherence of enterohemorrhagic *Escherichia coli* O157:H7 to HeLa cells. *Infect. Immun.*. 71(9):4985-4995.

Ukuku, D. O., and Fett, W. F. 2002. Relationship of cell surface charge and hydrophobicity to strength of attachment of bacteria to cantaloupe rind. *J. Food Prot*. 65:1093-1099.

Uhich, G. A., J. E. Keen, and R. O. Elder. 2002. Variations in the *csgD* promoter of *Escherichia coli* O157:H7 associated with increased virulence in mice and increased invasion of HEp-2 cells. *Infect. Immun*. 70:395-399.

Uhlich, G. A., J. E. Keen, and R. O. Elder. 2001. Mutations in the *csgD* promoter associated with variations in curli expression in certain strains of *Escherichia coli* O157:H7. *Appl. Environ. Microbiol*. 76:2367-2370.

USDA Economic Research Service. 1997. Food safety concerns for US fruits and vegetables. *Agr. Outlook.*

Van der Mei, H. C., M. Rosenberg, and H. J. Busscher. 1991. Assessment of microbial cell surface hydrophobicity. P. 263-288. In N. Mozes, P. S. Handley, H. J. Busscher, and P. G. Rouxhet (ed.), Microbial cell surface analysis. VCH, New York.

Van Loosdrecht, M. C. M., J. Lyklema, W. Norde, G. Scharaa, and A. J. B. Zehnder, 1987a. The role of bacterial cell wall hydrophobicity in adhesion. *Appl. Environ. Microbiol*. 53:1893-1897.

Van Loosdrecht, M. C. M., J. Lyklema, W. Norde, G. Scharaa, and A. J. B. Zehnder, 1987b. Electrophoretic mobility and hydrophobicity as a measuer to predict the initial step of bacterial adhesion. *Appl. Environ. Microbiol*. 53:1898-1900.

Wang, G., and M.P. Doyle. 1998. Survival of Enterohemorrhagic *Escherichia coli* O157:H7 in water. *J. Food Prot*. 61:662-667.

Wang, G., C. G. Clark, and F. G. Rodgers. 2002. Detection of *Escherichia coli* of the genes encoding the major virulence factors, the genes defining the O157:H7 serotype, and components of the type 2 shiga toxin family by multiplex PCR. *J. Clinical Microbiol*. 40:3613-3619.

Wang, L., and P. R. Reeves. 1998. Organization of *Escherichia coli* O157 O antigen gene cluster and identification of its specific genes. *Infect. Immun*. 66:3545-3551.

Willshaw, G. A., Scotland, S. M., and Rowe, B. 1997. Vero-cytotoxin-producing *E. coli*, p. 421-448. *In* M. Sussman (ed.), *Escherichia coli*: mechanisms of virulence. Cambridge University Press, United Kingdom.

Woody, J. M., R. A. Walsh, S. Doores, W. R. Henning, R. A. Wilson, and S. J. Knabel. 2000. Role of bacterial association and penetration on destruction of *Escherichia coli* O157:H7 in beef tissue by high pH. *J. Food Prot*. 63(1):3-11.

Zhao, T., M. P. Doyle, J. Shere, and L. Garber. 1995. Prevalence of Enterohemorrhagic *Escherichia coli* O157:H7 in a survey of dairy herds. *Appl. Environ. Microbiol*. 61:1290

CHAPTER #3:

Hydrophobicity, Cell Charge and Ability to Attach to Lettuce of *Escherichia coli* O157:H7 as Affected by Curli Production.

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Abstract:

Over the past decade, *Escherichia coli* O157:H7 has been increasingly responsible for foodborne outbreaks associated with produce. The removal of pathogens on produce surfaces has proven difficult. A better understanding of the mechanisms of bacterial attachment could lead to better risk reduction strategies. Some *E. coli* O157:H7 strains can produce curli under environmentally adverse conditions. Presence of curli is important in biofilm formation, and may affect the cells physicochemical properties (hydrophobicity, cell charge), and attachment ability. Curli producing (ATCC 43894+, ATCC 43895+, 0018+) and non-producing (ATCC 43894-, ATCC 43985-, 0018-) strains of *E. coli* O157:H7 were surveyed for their hydrophobicity and cell charge using hydrophobic interaction chromatography, and electrostatic interaction chromatography techniques. Iceberg lettuce squares (2 x 2 cm) were inoculated with *E. coli* O157:H7 (~7 log CFU/square), dried in a laminar flow hood, and rinsed twice with sterile de-ionized water. Strips (2 mm wide) of each cut edge of the lettuce were aseptically removed. Cut-edge and whole-leaf samples were homogenized and spiral plated onto Luria-Bertani Agar, supplemented with nalidixic acid (50 ppm), to assess levels of bacteria that remained on the lettuce leaf after rinsing. All strains, regardless of curli expression, attached preferentially to the cut edge lettuce ($p<0.05$). The 0018 strain which produced curli, attached significantly greater to iceberg lettuce than 0018 that do not produce curli ($p<0.05$), However no differences in attachment to lettuce were observed between curli

producing and non-producing strains 43894 and 43895. Cells producing curli were significantly more hydrophobic ($p<0.01$), however, no association with lettuce attachment was observed. Surface charge did not differ between strains. Results indicate that overall hydrophobicity and cell charge among strains of *E. coli* O157:H7 do not influence attachment to iceberg lettuce surfaces.

Introduction:

Escherichia coli O157:H7 became widely recognized as a foodborne pathogen in 1982 when it was linked to gastrointestinal illness caused by consumption of undercooked beef. Once thought to be primarily a pathogen associated with beef, *E. coli* O157:H7 has been implicated in several foodborne disease outbreaks associated with produce in the past decade. Between 1982 and 2002, 21% of all *E. coli* O157:H7 outbreaks were linked to produce *(29)*. Lettuce is the most common vegetable associated with *E. coli* O157:H7 produce outbreaks (34%) *(29)*.

Bacterial attachment is thought to be the first step in contamination of food products. If attachment did not occur, then an environment favorable to bacterial growth would never become established *(8)*. *E. coli* O157:H7 readily attaches to various produce surfaces including lettuce, apples, sprouts and cantaloupe *(7, 16, 17, 38, 41-45, 49)*. The removal of pathogens which may be present on produce surfaces has proven difficult. Research is being conducted to develop rinsing agents and sanitizers that will aid in removal of bacteria from produce surfaces. Such products may be used in packing houses before the product reaches the consumer market or as a rinse agent available for consumers that could be used in the home. This approach is essential to the future of produce safety; however, research needs to be conducted to provide a better understanding behind the cellular mechanisms of bacterial attachment. Specific cellular appendages present on the *E. coli* O157:H7 cell may influence bacterial attachment to various surfaces. If these attachment mechanisms were better understood, pre and post harvest prevention strategies or sanitation agents may be easier to develop.

Curli structures present on the surface of *E. coli* O157:H7 may affect the cells adhesion to produce surfaces. Curli are very thin, coiled extracellular structures expressed on the surface of most *E. coli* and *Salmonella entercia* strains which bind fibronectin and other proteins *(9, 24, 32)*. The components of these structures are highly conserved between the two species *(32)*. Curli are encoded by a conserved cluster of genes (*csgABCDEFG*) which are regulated by environmental conditions *(6)*. Some *E. coli* O157:H7 strains are unable to produce curli due to a mutation in the *csgD* promoter region *(10, 47, 48)*. However, Uhilch et al. *(47, 48)* revealed that the ATCC 93894 and ATCC 93895 strains do produce curli, and these strains do not contain the *csgD* mutation.

Curli expression is influenced by environmental factors such as temperature, osmolarity, and stationary phase growth *(1, 4, 21, 24, 28)*. *Escherichia coli* cells can express curli at low temperatures (22-26°C) but not at the cells' optimum growth temperature (35-37°) *(1, 21, 24, 28)*. Under stressful environmental conditions curli may be expressed as a survival mechanism.

Curli have been shown to mediate the attachment of cells to various surfaces. Research suggests that curli-producing *Salmonella* Typhimurium and curli-producing *E. coli* O157:H7 strains preferentially attach to intestinal cell lines over those strains which do not produce curli *(18, 40)*. Several curli producing Enterohemorrhagic strains of *E. coli* , have also exhibited an increased ability to adhere to thermonox, glass coverslips, polystyrene, and stainless steel coupons *(10, 25, 27)*. Curli also plays a role in the formation of biofilms *(33, 36, 37)*. Biofilms may allow the cell to evade sanitation steps *(35)*. This is important when considering sanitation programs of produce packing houses

and food processing environments. Further research regarding the adhesion of curli producing bacteria to produce or plant surfaces is essential.

The presence of extracellular structures (ex. fimbriae and lipopolysaccharides) can influence a cell's physicochemical properties *(13, 34)*. These bacterial properties (hydrophobicity and cell charge) may be an important factor associated with attachment to produce surfaces. The expression of curli may influence these cell properties. Hydrophobicity is positively correlated with an increase in the ability of a cell to adhere to abiotic (stainless steel, polystyrene, and rubber) and biotic surfaces (soybean leaves, beef fat, meat, cantaloupe) *(3, 5, 11, 13, 19, 25, 49, 50)*. Other research has found that the hydrophobicity does not affect attachment to apple, lettuce, and beef muscle *(11, 16, 19)*.

Less is known about the influence of cell charge on attachment. Cells with a strong negative charge attach preferentially to stainless steel and beef muscle *(5, 11)*. However other research has shown that cell charge has no effect on attachment to stainless steel, beef, apples and lettuce *(13, 19, 50)*. A more complete understanding behind the overall hydrophobicity and charge of bacteria, as well as factors which influence these properties may provide better understanding behind attachment mechanisms.

The objectives of this study were to determine whether curli producing or non-producing strains of *E. coli* O157:H7 attach preferentially to cut edge and/or whole leaf lettuce surfaces. The effect of curli expression on the cells physicochemical properties (hydrophobicity, and cell surface charge) was evaluated. Any correlation between the

hydrophobicity and cell charge of the *E. coli* O157:H7 strain and its ability to attach to lettuce surfaces was determined.

Materials and Methods:

<u>Bacterial cultures:</u>

Strains of *E. coli* O157:H7 deficient in production of curli (ATCC 43894-, ATCC 43985-, 0018-) and their corresponding curli-producing strains (43894+, 43895+, 0018+) were used in this study. The ATCC strains were provided by Dr. G.A. Uhlich (United States Department of Agriculture, Agriculture Research Service, Wyndmoor, PA, USA). The 0018 strains (calf isolate) were provided by Dr. Larry Beuchat (Center for Food Safety and Department of Food Science and Technology, University of Georgia, Griffin, GA, USA). Cultures were confirmed to be *E. coli* O157:H7 by plating on Sorbitol MacConkey Agar No 3 (SMAC) (Oxoid Ltd, Busingstoke, Hampshire, England) and using RIM *E. coli* O157:H7 latex agglutination test (Remel, Lenexa, KS). Confirmation of curli phenotype was completed by plating strains onto Congo red indicator (CRI) plates (Luria Bertani Agar (LBA) (BD, Franklin Lakes, NJ) supplemented with 40µg/ml congo red dye and 20µg/ml Coomassie brilliant blue dye). Curli are characteristic in their ability to bind congo red dye producing red colonies. Curli negative colonies are unable to bind congo red and produce white or colorless colonies. These are common methods to identify curli production among strains *(6, 12, 14, 15, 35, 37, 47, 48)*. Bacterial cultures were made nalidixic acid resistant by plating onto LBA with increasing concentrations (5, 10, 25, 40, and 50ppm) to achieve resistance to a final concentration of 50 ppm (LBAN).

<u>Inoculum:</u>

Bacterial cultures were stored in a 30% glycerol, LB broth solution at –80°C. Cells were activated by three successive 24 hour transfers in LB. Methods described by Ryu et al. *(36, 37)* were used to create conditions more favorable to curli production. Following activation, 24-hr cultures of each strain were serially diluted to 10^{-5} cfu/ml using 0.1% peptone, and spread plated onto LBAN agar. Plates were incubated at 22°C for 3 days to enhance expression of curli. Following the three day incubation, one colony for each strain was streaked onto congo red agar for confirmation of curli presence/absence. Using a sterile plastic bent rod, cells were collected from plates and resuspended into 500ml of sterile de-ionized water (SDW) to achieve approximately 7.0 log cfu/ml of inoculum. Lettuce samples were inoculated immediately following preparation of inoculum.

Lettuce Attachment studies:

Iceberg lettuce was purchased from a local grocery store. Exterior leaves of the lettuce were aseptically removed and discarded. Lettuce was cut into 2x2 cm squares with a sterile scalpel under a laminar flow hood to prevent contamination. One sample consisted of three pieces of lettuce. Two samples (six lettuce squares) were suspended in each inoculum: 0018+, 0018-, 43894+, 43894-, 43895+, 43895-, and sterile de-ionized water (SDW) (un-inoculated control). These seven samples were incubated for 24±2 hours at 4°C. Immediately following inoculation, lettuce squares were allowed to dry on a sterile drying rack in laminar flow hood for 30 minutes prior to sampling. One sample (three pieces of lettuce) from each inoculum was then analyzed immediately and considered the un-rinsed sample, and another sample was analyzed following two successive rinses in 100ml SDW for one min each and considered the rinsed sample.

Each rinse solution (rinse #1 and rinse #2) was enumerated for concentration of *E. coli* O157:H7 following the rinse.

During sampling, bacterial concentrations present on the cut edge and whole leaf were analyzed. The outermost 0.2 cm of the lettuce leaf (approximately 0.30 g) was excised on all four sides with a sterile scalpel (all three pieces). These were considered the cut edge samples, and the remaining whole portion of the leaf (approximately 0.70g) was considered the whole leaf samples. Samples were analyzed separately and referred to as "cut edge" and "whole leaf".

Each sample was then added to a 400ml Fisherbrand® filtered stomacher bag (Fisher, Hampton, NH) and homogenized in Masticator (IUL Instruments, Barcelona, Spain) with 99ml of peptone water. Homogenate was diluted and spiral plated on LBAN using an Autoplate© 4000 (Spiral Biotech, Norwood, MA). Plates were incubated for 48 hours at 37°C. Following incubation, plates were counted and one colony from each set of duplicate plates was analyzed using RIM *E. coli* O157:H7 latex agglutination test for confirmation of strain, and plated onto CRI for confirmation of curli production.

<u>Hydrophobic interaction chromatography:</u>

Hydrophobic interaction chromatography (HIC) was used to determine the cells' hydrophobicity, methods were similar to those used by Ukuku and Fett *(49)* and several other researchers *(11, 23, 26)*. Empty Polyprep columns® (Biorad, Hercules, CA) were packed with 2 bed volumes of Octyl-Sepahrose® gel CL-4B (Sigma, Saint Louis, MO) and equilibrated overnight at 4°C with 1M ammonium sulfate. Prior to use, columns were rinsed with 10ml of 1M ammonium sulfate. Bacterial cultures were prepared as described in lettuce attachment studies.

A portion (0.1ml) of bacterial cells was added to each column with 0.4ml of 2 M ammonium sulfates. Cells were eluted with 9.5 ml of 2 M ammonium sulfate. Inoculum and eluted bacteria were analyzed separately by the spread plate method on LBAN. Plates were incubated for 48 hours at 35°C. Relative hydrophobicity is presented as the number of bacteria retained in the columns (*g*) over the number of bacteria in the original cell suspension (*e*) or *g/e*. Three replicates of each trial were completed.

Electrostatic interaction chromatography (EISC):

Cell surface charge of bacterial cultures was determined similarly to the previously described methods *(11, 26, 49)*. However, Prepacked Polyprep® Ion exchange columns pre-filled with AG 1-X8 resin, 100-200 mesh Chloride form and Prepacked Polyprep® Ion exchange columns pre-filled with AG 50W-X8 resin, 100-200 mesh, Hydrogen form(BioRad, Hercules, CA) were used. Cultures were prepared as described in attachment studies.

ESIC columns were rinsed w/ 10 ml of 0.02 M NaPO4 buffer prior to the addition of bacteria. An aliquot (0.1 ml sample) of washed bacterial suspension was loaded onto the surface of the column. 0.4 ml of 0.02 M $NaPO_4$ buffer was added to the column after the bacteria. Columns were eluted with 9.5 ml of 0.02 M $NaPO_4$ buffer, and the eluate was collected. Bacterial populations were determined from the original cell suspension and the eluted suspension by spread plating onto LBAN. The number of bacteria bound to the column was determined by the difference between the original bacterial population and the eluted bacterial population. Results are expressed as *r/e*, where r is the number of bacteria retained in the columns and e is the number eluted. Three replicates of each trial were completed.

Statistical Analysis:

The effect of the strain (0018, 43894, 43895), and expression of curli (+, -) on the ability of the cell to adhere to cut edge and whole leaf surfaces for both rinsed and unrinsed samples was compared by two way ANOVA separately (two different analyses, one for cut edge, one for whole leaf). The effect of rinsing (unrinsed, rinsed), and the expression of curli (+, -) on the ability of cells to adhere to cut edge and whole leaf surfaces was also completed by two way ANOVA method.

The effect of strain (0018, 43894, 43895), and expression of curli (+, -) on the cells hydrophobicity, positive and negative cell charges was also analyzed by two way ANOVA separately (three different analysis, hydrophobicity, positive, and negative cell charge). The relationship between each cell physicochemical characteristic and the amount of bacteria attached to cut edge and whole leaf structures was analyzed by linear regression.

All statistical analysis were performed by the statistical consulting center at Virginia Polytechnic Institute and State University using SAS statistical software (SAS institute Inc., Cary, N.C.). Tukey's HSD was performed to determine significant differences between means.

Results:

The inoculum level for the three strains was consistent between strains and curli phenotype, ranging between 7.1 and 7.5 log CFU/ml. Un-inoculated lettuce controls had no growth on LBN or CRI plates for all experiments. Using SMAC and *E. coli* O157:H7 RIM Latex agglutination tests, *E. coli* O157:H7 was confirmed on all LBN plates from

sampled lettuce. All curli producing strains formed red colonies and all curli non-producing strains produced white colonies on CRI plates consistently.

<u>Attachment of *E. coli* O157:H7 curli producing and non-producing strains to lettuce surfaces.</u>

Following the removal of lettuce leaves from the inoculum, inoculated leaves were air dried in a laminar flow through hood on a sterile drying rack for 30 minutes before analysis. The concentration (log CFU/g) of cells attached to the surface of un-rinsed cut leaf and whole edge lettuce samples was determined immediately following the drying period. There were no significant differences in attachment between the curli producing and non-producing strains (0018, 93894, 93895) to the unrinsed cut edge and whole leaf lettuce samples (Table 3.1). All *E. coli* O157:H7, showed no preference in attachment to cut edge or whole leaf portion of the lettuce prior to rinsing (Table 3.1).

The concentration of *E. coli* O157:H7 cells enumerated in rinse #1 and rinse #2 following the rinse step was determined For curli producing and non-producing 0018 strains, there was approximately a 0.8-log reduction between bacterial concentrations recovered from rinse #1 to rinse #2 (Figure 3.1). For curli producing and non-producing 43894 strains, there was approximately a 0.8-log reduction and a 1.2-log reduction (Figure 3.2). Finally, for curli producing and non-producing 43895 strains, there was approximately a 1.5-log reduction and a 0.4-log reduction (Figure 3.2). The concentrations of bacteria recovered in the rinses, however, did not affect the concentration of cells attached to the rinsed lettuce (Figures 3.1 and 3.2).

In all cases bacterial strains favorably attached to the cut edges of the lettuce ($p>0.05$) following the rinse steps. Prior to rinsing there was no significant difference between cut edge and whole leaf attachment. Curli producing *E. coli* O157:H7 0018 had

significantly greater attachment to cut edge and whole leaf lettuce samples than curli non-producing phenotype as shown in Table 3.1 ($p<0.05$). However, no significant differences in the attachment of curli producing and non-producing strains 48394 and 48395 to cut edge and whole leaf lettuce samples were seen. The curli non-producing strains exhibited greater attachment to both cut edge and whole leaf lettuce samples even though the difference is not significant (Table 3.1).

In most cases, there were no significant differences when comparing unrinsed cut with rinsed cut for the same strain and curli phenotype. However, attachment of curli non-producing 43894, and curli producing 43895 was significantly different between unrinsed and rinsed cut edge and whole leaf lettuce samples.

Relationship of physicochemical properties to attachment.

Escherichia coli O157:H7 strains exhibited low hydrophobic characteristics. Strain type (0018, 93894, and 93895) had no significant effect on hydrophobicity. All curli producing strains were significantly more hydrophobic than non-curli producing strains as shown in Table 3.2. Both strain type (0018, 93894, and 93895) and curli production had no significant effect on positive or negative cell charge (Table 3.2). There was no correlation in the cell's physicochemical properties (hydrophobicity, cell charge) and ability to attach to cut edge or whole leaf lettuces surfaces.

Discussion:

Bacterial attachment to produce surfaces is a poorly understood process. It may be influenced by specific factors (fimbriae, lipopolysaccharide, etc.) or non-specific factors (cell surface charge and hydrophobicity). This study investigated the effect of

curli (specific factor) as well as hydrophobicity and cell charge (non-specific factors) on the attachment of *E. coli* O157:H7 to lettuce surfaces.

Physicochemical properties:

The presence of extra cellular appendages on surfaces of bacteria are thought to influence the cells' physicochemical properties (hydrophobicity and cell charge). Plants and bacteria generally have a negative surface potential, which results in electrostatic repulsion between the two surfaces *(49)*. Surface appendages such as curli may help the bacteria to overcome this repulsion allowing specific attachment to plant surfaces to occur *(31, 49)*

Hydrophobic properties vary between strains and serotypes of bacteria *(20, 30)*. Wild type *E. coli* O157:H7 has been found to have a relatively low surface hydrophobicity with data ranging between 0.202 and 0.233 when analyzed using the hydrophobic interaction chromatography (HIC) method *(11, 39, 49)*. When comparing curli producing and non-producing strains using the HIC method, it was found that curli producing strains consistently had a hydrophobicity of between 0.2-0.28; these values are similar to previous mentioned research. However, the curli non-producing strains were significantly less hydrophobic, ranging between 0.03-0.07. *Escherichia coli* strains previously evaluated for their hydrophobic characteristics may or may not have produced curli. Most *E. coli* O157:H7 are unable to produce curli due to a base pair change in the *csgD* promoter region *(47)*. Curli is made up of CsgA monomers expressed on the cells surface. These monomers may by hydrophobic, which would explain why the expression of curli increased the cells hydrophobicity.

Escherichia coli classically are predominantly negative in charge. In the study by Ukuku and Fett, all three *E. coli* strains analyzed (ATCC 25922, O157:H7 SEA13B88, and O157:H7 Oklahoma) had a much weaker positive charge (0.12-0.16) than negative charges (1.48-1.62) when analyzed using electrostatic interaction chromatography techniques *(49)*. Dickson and Koohmaraie also analyzed several *E. coli* strains using the ESIC technique and found similar results, reporting overall positive and negative charges being 0.16 and 1.33 *(11)*. The O157:H7 serotype of *E. coli* exhibits a lower negative cell charge than other shiga-toxigenic *E. coli* (STEC) *(30)*. Which may explain why *E. coli* O157:H7 strains tend to readily attach to surfaces over some other *E. coli (17)*. No overall significant differences were seen in the positive (0.16) and negative (1.19) cell charges between strains and curli phenotype of *E. coli* O157. Our results indicated that the presence of curli did not affect the cells charge of *E. coli* O157:H7

Attachment to cut edge and whole leaf lettuce portions:

Escherichia coli O157:H7 strains have previously been reported to attach preferentially to cut edges of lettuce leaves verses whole lettuce surface *(16, 41, 43)*. Our studies also found that all curli producing and non-producing *E. coli* O157:H7 strains used attached preferentially to cut edge over the whole leaf lettuce samples.

It was hypothesized that the expression of curli would enhance the attachment of *E. coli* O157:H7 to lettuce surfaces. Curli producing *E. coli* O157:H7 strain 0018 attached to cut edge and whole leaf portions of lettuce significantly more than curli non-producing strains. However, curli producing 43894 and 43895 strains attached to cut and whole leaf portions of lettuce consistently less than curli non-producing strains, although this difference was not significant. Attachment of *E. coli* O157:H7 may not be affected

by curli expression. Recently, Jeter and Matthysse reported that when a plasmid carrying *csgA-G* was inserted into a non adhering laboratory *E. coli* K12 strain, the strain was then able to adhere to alfalfa sprouts *(17)*. However, when mutations in the *csg* operon of *E. coli* O157:H7 were made, there was no difference in attachment *(17)*. There is evidence that other extracellular outer membrane proteins may be involved in attachment of bacteria to produce surfaces. Torres et al. found that mutations in the *E. coli* O157:H7 *ompA* gene significantly reduced the cells ability to attach to alfalfa sprouts *(45)*. This outer membrane protein is reported to have the most considerable effect on attachment to food plant surfaces.

More than one mechanism may be important for binding pathogenic strains. Even though curli alone may not play a role in attachment to produce surfaces, they have been identified as important factors influencing the development of biofilms *(32, 36, 37, 46)*. Production of biofilms by bacteria increases their able to persist in the environment. Bacterial biofilms have been observed on several leafy vegetables *(22)*. Curli producing bacteria may enhance attachment of bacterial surfaces, solely by their relationship with biofilm production.

No correlation was found between the increased hydrophobicity of the curli producing strains and attachment to lettuce. The surfaces of lettuce and other produce items are covered by a hydrophobic waxy cuticle. It is suspected that hydrophobic bacteria would preferentially attach to those surfaces. One study found that *E. coli* preferentially attached to the hydrophobic waxy cuticle that covers the surface of apples *(7)*. Hassan and Frank *(16)* concluded that the less hydrophobic cells attached better to apples and lettuce. Although the presence of curli increased the cells' hydrophobicity

there was no increase in the cells attachment to lettuce. Hydrophobic interactions may not be important in mediating attachment to produce surfaces.

Continued rinsing of lettuce ineffective at removal of cells from lettuce.

Methods used in this study were adapted from other lettuce attachment studies. Common protocol involves inoculating lettuce for an extended period time (18-24 hrs) to simulate field conditions or rain; followed by two consecutive rinses in SDW to remove any "loosely attaching bacteria" *(16, 41, 42)*. Attachment between microorganisms and plant surfaces may occur in two phases: a primary, initial attachment that is reversible and unspecific; followed by a highly specific irreversible attachment *(31)*. The initial research plan was to quantify the amounts of bacteria which were "loosely attaching" verses "tightly attaching" similar to a previous study *(49)*. However, there was no reduction in concentrations of bacteria which were attached to the lettuce following two rinses in SDW. Interestingly, when enumerating the separate rinse water samples, bacterial levels were very similar to the bacterial counts on the lettuce following the rinse step. The largest reduction of attachment on lettuce following the rinse step was approximately 1-log. No correlation between loosely and tightly attached bacteria could be made.

The lengthy inoculation time may have influenced the degree of attachment as well as encouraged uptake of the bacteria into the cells making them harder to remove, or more tightly attached. Sprouts inoculated for one day with 10^3 cfu/ml *E. coli* O157:H7, had 3.2-3.8 log CFU/ml attached following two consecutive rinses with 5ml of buffer *(17)*. That study did not enumerate their rinses. Inoculum levels for our study were approximately 7.0 log CFU/ml and bacteria attached following rinses were between 5.5-

6.5 log CFU/ml respectively. Another study found that *Escherichia coli* O157:H7 were easily removed from alfalfa sprouts following three separate consecutive washes with sterile water *(2)*. Seo and Frank found that *E. coli* O157:H7 was able to penetrate through the cut edges of the lettuces into the leaf tissue *(38)*. Research using a shorter inoculation time may not allow for penetration of the bacteria. This may be a more appropriate method for determining specific attachment mechanisms and removal. Additionally, the use of intact lettuce leaves may prevent uptake of the bacteria.

While the presence of curli increases the ability of *E. coli* O157:H7 to form biofilms, this research indicates that curli are not essential for attachment to lettuce surfaces. Curli may be able to affect the cell's hydrophobicity, but have no influence on cell charge. The effect of physicochemical properties on a cells' attachment to lettuce was not elucidated with this research. Attachment of bacteria to various surfaces has proved to be a complicated one. This area of food safety research is essential. A more thorough understanding behind attachment may aid in the development of pre and post harvest interventions which could reduce the potential for bacterial contamination. Continued research should be performed to look at the attachment mechanisms of *E. coli* O157:H7 and other foodborne pathogens to various surfaces.

References:

1. Arnqvist, A., A. Olsen, J. Pfeifer, D. G. Russell, and S. Normark. 1992. The Crl protein activates cryptic genes for curli formation and fibronectin binding in *Escherichia coli* HB101. *Mol Microbiol* 6**:**2443-2452.

2. Barak, J. D., L. Gorski, P. Naraghi-Arani, and A. O. Charkowski. 2005. *Salmonella enterica* virulence genes are required for bacterial attachment to plant tissue. *Appl Environ Microbiol* 71**:**5685-5691.

3. Benito, Y., C. Pin, M. Luisa Martin, M. Luisa Farcia, M. Delores Selagas, and C. Casas. 1997. Cell surface hydrophobicity and attachment of pathogenic and spoilage bacteria to meat surfaces. *Meat Science* 45.

4. Bougdour, A., C. Lelong, and J. Geiselmann. 2004. Crl, a low temperature-induced protein in *Escherichia coli* that binds directly to the stationary phase sigma subunit of RNA polymerase. *J Biol Chem* 279**:**19540-19550.

5. Briandet, R., T. Meylheuc, C. Maher, and M. N. Bellon-Fontaine. 1999. *Listeria monocytogenes* Scott A: cell surface charge, hydrophobicity, and electron donor and acceptor characteristics under different environmental growth conditions. *Appl Environ Microbiol* 65**:**5328-5333.

6. Brown, P. K., C. M. Dozois, C. A. Nickerson, A. Zuppardo, J. Terlonge, and R. Curtiss, 3rd. 2001. MlrA, a novel regulator of curli (AgF) and extracellular matrix synthesis by *Escherichia coli* and *Salmonella enterica* serovar Typhimurium. *Mol Microbiol* 41**:**349-363.

7. Burnett, S. L., J. Chen, and L. R. Beuchat. 2000. Attachment of *Escherichia coli* O157:H7 to the surfaces and internal structures of apples as detected by confocal scanning laser microscopy. *Appl Environ Microbiol* 66**:**4679-4687.

8. Cabedo, L., J.N. Sofos, G.R. Schmidt, and G.C.Smith. 1997. Attachment of *Escherichia coli* O157:H7 and other bacterial cells grown in two media to beef adipose and muscle tissue. *J Food Prot* 60.

9. Collinson, S. K., S. C. Clouthier, J. L. Doran, P. A. Banser, and W. W. Kay. 1996. *Salmonella enteritidis* agfBAC operon encoding thin, aggregative fimbriae. *J Bacteriol* 178**:**662-667.

10. Cookson, A. L., W. A. Cooley, and M. J. Woodward. 2002. The role of type 1 and curli fimbriae of Shiga toxin-producing *Escherichia coli* in adherence to abiotic surfaces. *Int J Med Microbiol* 292**:**195-205.

11. Dickson, J. S., and M. Koohmaraie. 1989. Cell surface charge characteristics and their relationship to bacterial attachment to meat surfaces. *Appl Environ Microbiol* 55**:**832-836.

12. Evans, D. G., D. J. Evans, Jr., and W. Tjoa. 1977. Hemagglutination of human group A erythrocytes by enterotoxigenic *Escherichia coli* isolated from adults with diarrhea: correlation with colonization factor. *Infect Immun* 18**:**330-337.

13. Fett, W. F. 1985. Relationship of bacterial cell surgace hydrophobicity and charge to pahogenicity, physiologic race and immobilization in attached soybean leaves. *Physiol. Biochem.* 75**:**1414-1418.

14. Hammar, M., A. Arnqvist, Z. Bian, A. Olsen, and S. Normark. 1995. Expression of two csg operons is required for production of fibronectin- and congo red-binding curli polymers in *Escherichia coli* K-12. *Mol Microbiol* 18**:**661-670.

15. Hammar, M., Z. Bian, and S. Normark. 1996. Nucleator-dependent intercellular assembly of adhesive curli organelles in *Escherichia coli*. *Proc Natl Acad Sci U S A* 93**:**6562-6566.

16. Hassan, A. N., and J. F. Frank. 2004. Attachment of *Escherichia coli* O157:H7 grown in tryptic soy broth and nutrient broth to apple and lettuce surfaces as related to cell hydrophobicity, surface charge, and capsule production. *Int J Food Microbiol* 96**:**103-109.

17. Jeter, C., and A. G. Matthysse. 2005. Characterization of the binding of diarrheagenic strains of *E. coli* to plant surfaces and the role of curli in the interaction of the bacteria with alfalfa sprouts. *Mol Plant Microbe Interact* 18**:**1235-1242.

18. Kim, S.-H. K. a. Y.-H. 2004. *Escherichia coli* O157:H7 adherence to HEp-2 cells is implicated with curli expression and outer membrane integrity. *J Veterinary Science* 5**:**119-124.

19. Li, Y., L.A. McLandsborough. 1999. The effects of the surface charge adn hydrophobicity of *Escherichia coli* on its adhesion to beef muscle. *Int J Food Microbiol* 53.

20. Lytle, D. A., E. W. Rice, C. H. Johnson, and K. R. Fox. 1999. Electrophoretic mobilities of *Escherichia coli* O157:H7 and wild-type Escherichia coli strains. *Appl Environ Microbiol* 65**:**3222-3225.

21. Maurer, J. J., T. P. Brown, W. L. Steffens, and S. G. Thayer. 1998. The occurrence of ambient temperature-regulated adhesins, curli, and the temperature-sensitive hemagglutinin tsh among avian *Escherichia coli*. *Avian Dis* 42**:**106-118.

22. Morris, C. E., J-M, Monier, and M-A. Jaques. 1997. Methods for observing microbial biofilms directly on leaf surfaces and recovering them for isolations of culturable microorganisms. *Appl Environ Microbiol* 63**:**1570-1576.

23. Mozes, N. a. P. G. R. 1987. Methods for measuring hydrophobicity of microorganisms. *J Microbiol Methods* 6**:**99-112.

24. Olsen, A., A. Jonsson, and S. Normark. 1989. Fibronectin binding mediated by a novel class of surface organelles on *Escherichia coli. Nature* 338**:**652-655.

25. Pawar, D. M., M. L. Rossman, and J. Chen. 2005. Role of curli fimbriae in mediating the cells of enterohaemorrhagic *Escherichia coli* to attach to abiotic surfaces. *J Appl Microbiol* 99**:**418-425.

26. Pederson, K. 1980. Electrostatic interaction chromatography, a method for assaying the realtive surface charges of bacteria. *FEMS Microbiol Lett* 22**:**295-298

27. Prigent-Combaret, C., G. Prensier, T. T. Le Thi, O. Vidal, P. Lejeune, and C. Dorel. 2000. Developmental pathway for biofilm formation in curli-producing *Escherichia coli* strains: role of flagella, curli and colanic acid. *Environ Microbiol* 2**:**450-464.

28. Provence, D. L., and R. Curtiss, 3rd. 1992. Role of crl in avian pathogenic *Escherichia coli*: a knockout mutation of crl does not affect hemagglutination activity, fibronectin binding, or Curli production. *Infect Immun* 60**:**4460-4467.

29. Rangel, J. M., P. H. Sparling, C. Crowe, P. M. Griffin, and D. L. Swerdlow. 2005. Epidemiology of *Escherichia coli* O157:H7 outbreaks, United States, 1982-2002. *Emerg Infect Dis* 11**:**603-609.

30. Rivas, L., N. Fegan, and G. A. Dykes. 2005. Physicochemical properties of Shiga toxigenic Escherichia coli. *J Appl Microbiol* 99**:**716-727.

31. Romantschuk, M., RL. Nurmiaho-Lassila, E. Roine, and A. Suoniemi. 1993. Pilus mediated adsorption of Pseudomonas syringae to the surface of host and non-host plant leaves. *J Gen Microbiol.* 139**:**2251-2260.

32. Romling, U., Z. Bian, M. Hammar, W. D. Sierralta, and S. Normark. 1998. Curli fibers are highly conserved between *Salmonella typhimurium* and *Escherichia coli* with respect to operon structure and regulation. *J Bacteriol* 180**:**722-731.

33. Romling, U., M. Rohde, A. Olsen, S. Normark, and J. Reinkoster. 2000. AgfD, the checkpoint of multicellular and aggregative behaviour in *Salmonella typhimurium* regulates at least two independent pathways. *Mol Microbiol* 36**:**10-23.

34. Rosenberg, M., D. Gutnick, and E. Rosenberg. 1980. Adherence of bacteria to hydrocarbons: a simple method for measuring cell-surface hydrophobicity. *FEMS Microbiol Lett* 9**:**29-33.

35. Ryu, J. H., and L. R. Beuchat. 2005. Biofilm formation by *Escherichia coli* O157:H7 on stainless steel: effect of exopolysaccharide and Curli production on its resistance to chlorine. *Appl Environ Microbiol* 71**:**247-254.

36. Ryu, J. H., H. Kim, and L. R. Beuchat. 2004. Attachment and biofilm formation by *Escherichia coli* O157:H7 on stainless steel as influenced by exopolysaccharide production, nutrient availability, and temperature. *J Food Prot* 67**:**2123-2131.

37. Ryu, J. H., H. Kim, J. F. Frank, and L. R. Beuchat. 2004. Attachment and biofilm formation on stainless steel by *Escherichia coli* O157:H7 as affected by curli production. *Lett Appl Microbiol* 39**:**359-362.

38. Seo, K. H., and J. F. Frank. 1999. Attachment of *Escherichia coli* O157:H7 to lettuce leaf surface and bacterial viability in response to chlorine treatment as demonstrated by using confocal scanning laser microscopy. *J Food Prot* 62**:**3-9.

39. Sherman, P., R. Soni, and m. Karmali. 1987. Surface properties of the vero cytotoxin-producing Escherichia coli O157:H7. *Infect Immun* 55**:**1824-1829.

40. Sukupolvi, S., R. G. Lorenz, J. I. Gordon, Z. Bian, J. D. Pfeifer, S. J. Normark, and M. Rhen. 1997. Expression of thin aggregative fimbriae promotes interaction of *Salmonella typhimurium* SR-11 with mouse small intestinal epithelial cells. *Infect Immun* 65**:**5320-5325.

41. Takeuchi, K., and J. F. Frank. 2000. Penetration of *Escherichia coli* O157:H7 into lettuce tissues as affected by inoculum size and temperature and the effect of chlorine treatment on cell viability. *J Food Prot* 63**:**434-440.

42. Takeuchi, K., and J. F. Frank. 2001. Direct microscopic observation of lettuce leaf decontamination with a prototype fruit and vegetable washing solution and 1% NaCl-NaHCO3. *J Food Prot* 64**:**1235-1239.

43. Takeuchi, K., and J. F. Frank. 2001. Quantitative determination of the role of lettuce leaf structures in protecting *Escherichia coli* O157:H7 from chlorine disinfection. *J Food Prot* 64**:**147-151.

44. Takeuchi, K., A. N. Hassan, and J. F. Frank. 2001. Penetration of *Escherichia coli* O157:H7 into lettuce as influenced by modified atmosphere and temperature. *J Food Prot* 64**:**1820-1823.

45. Torres, A. G., C. Jeter, W. Langley, and A. G. Matthysse. 2005. Differential binding of *Escherichia coli* O157:H7 to alfalfa, human epithelial cells, and plastic is mediated by a variety of surface structures. *Appl Environ Microbiol* 71**:**8008-8015.

46. Torres, A. G., and J. B. Kaper. 2003. Multiple elements controlling adherence of enterohemorrhagic *Escherichia coli* O157:H7 to HeLa cells. *Infect Immun* 71**:**4985-4995.

47. Uhlich, G. A., J. E. Keen, and R. O. Elder. 2001. Mutations in the csgD promoter associated with variations in curli expression in certain strains of *Escherichia coli* O157:H7. *Appl Environ Microbiol* 67**:**2367-2370.

48. Uhlich, G. A., J. E. Keen, and R. O. Elder. 2002. Variations in the csgD promoter of *Escherichia coli* O157:H7 associated with increased virulence in mice and increased invasion of HEp-2 cells. *Infect Immun* 70**:**395-399.

49. Ukuku, D. O., and W. F. Fett. 2002. Relationship of cell surface charge and hydrophobicity to strength of attachment of bacteria to cantaloupe rind. *J Food Prot* 65**:**1093-1099.

50. Van Loosdrecht, C. M. M., J. Lyklema., W. Norde, G. Scharaa, and A. J. B. Zehnder. 1987. Electrophoretic mobility and hydrophobicity as a measure to predict the initial step of bacterial adhesion. *Appl Environ Microbiol* 53**:**1898-1900.

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Table 3.1. Attachment (Log CFU/g) of curli producing (+) and non-producing (-) *E. coli* O157:H7 strains (0018, 43894, and 43895) to cut edge and whole leaf lettuce sections before (unrinsed) and after (rinsed) two successive 1 min rinses

		18		43894		43895	
Treatment	Lettuce Sample	(+)	(-)	(+)	(-)	(+)	(-)
Unrinsed	Cut Edge	6.80^{a}	6.20^{a}	6.39^{a}	7.38^{a}	6.12^{a}	6.20^{a}
	Whole leaf	6.60^{a}	6.07^{a}	6.00^{a}	7.08^{a}	5.89^{a}	6.07^{a}
Rinsed	Cut Edge	6.61$^{a.1}$	5.58^{a,2}	5.98^{a,1}	6.66^{a,1}	5.22^{a,1}	5.88^{a,1}
	Whole leaf	6.17$^{b.1}$	5.11^{b,2}	5.39^{b,1}	6.24^{b,1}	4.95^{b,1}	5.55^{b,1}

Statistical significance is indicated as follows: Individual treatments (unrinsed, rinsed) are analyzed separately:
Values with different superscript letters within columns are significantly different ($P \leq 0.05$).
Values with different superscript numbers within rows within the same strain (0018, 43894, and 43895) are significantly different ($p \leq 0.05$).

Table 3.2. Hydrophobicity and cell charge characteristics of curli producing (+) and non-producing (-) strains (0018, 43894, and 43895) of *E. coli* O157:H7. (n=3)

		18		43894		43895	
		(+)	(-)	(+)	(-)	(+)	(-)
Hydrophobicity (*g/e*)		0.2[a]	0.07[b]	0.2[a]	0.03[b]	0.28[a]	0.06[b]
Cell Charge (*r/e*)	(+)	0.09[a]	0.41[a]	0.05[a]	0.07[a]	0.35[a]	0.26[a]
	(-)	0.74[a]	1.17[a]	1.1[a]	0.98[a]	0.65[a]	1.41[a]

Values with different superscript letters in the same row are significantly different ($P<0.05$).

Cell charge: (+) denotes amount of positive charge; and (-) denotes amount of negative charge on cell surface.

Hydrophobicity is expressed as *g/e*:

g: number of bacteria retained in the column

e: number of bacteria in the original cell suspension.

Cell charge is expressed as *r/e*:

r: number of bacteria retained in the column

e: number of bacteria in the original cell suspension

Figure 3.1 Attachment (Log CFU/g) of *E. coli* O157:H7 0018 curli producing (+) and curli non-producing (-) strains (0018, 43894, 43895) to unrinsed and rinsed cut edge and whole leaf lettuce sections before and after two successive 1 min rinses (n=6).
Unrinsed: concentration of cells attached to lettuce surfaces following inoculation.
Rinse #1, 2: Concentration of cells left in the rinse wash following rinses.
Rinsed: concentration of cells attached to lettuce surfaces after the two rinses.

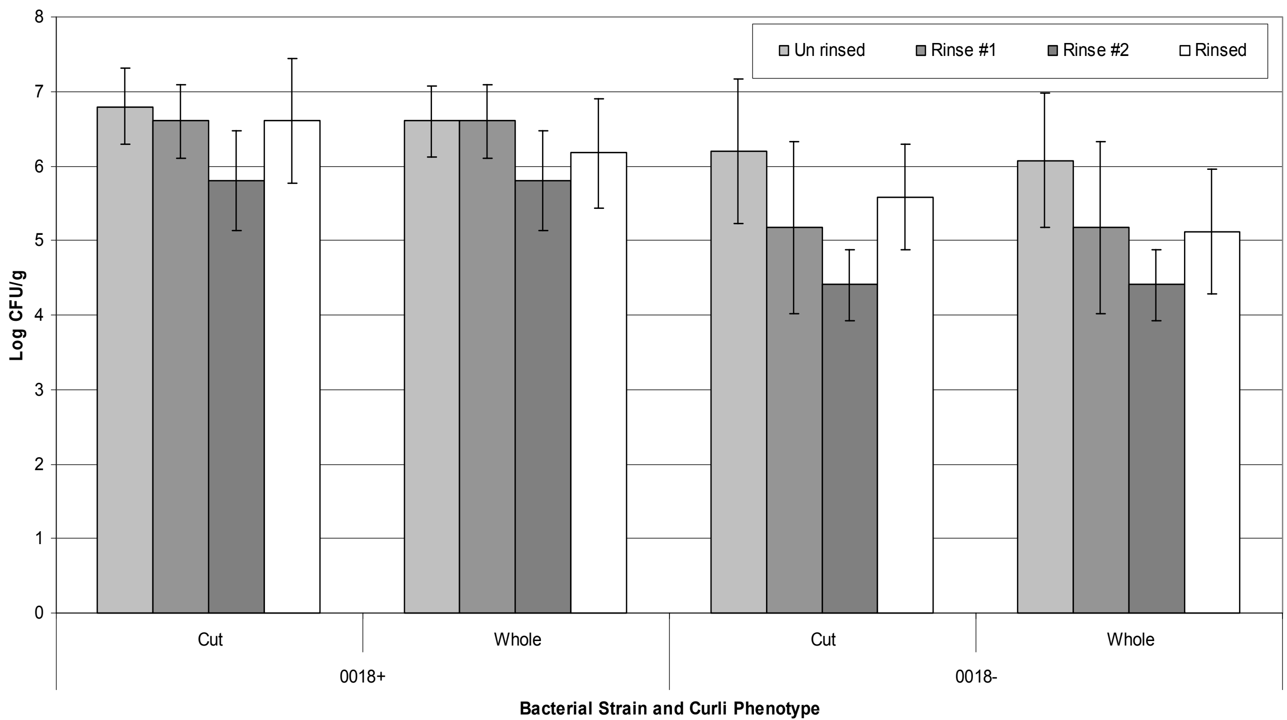
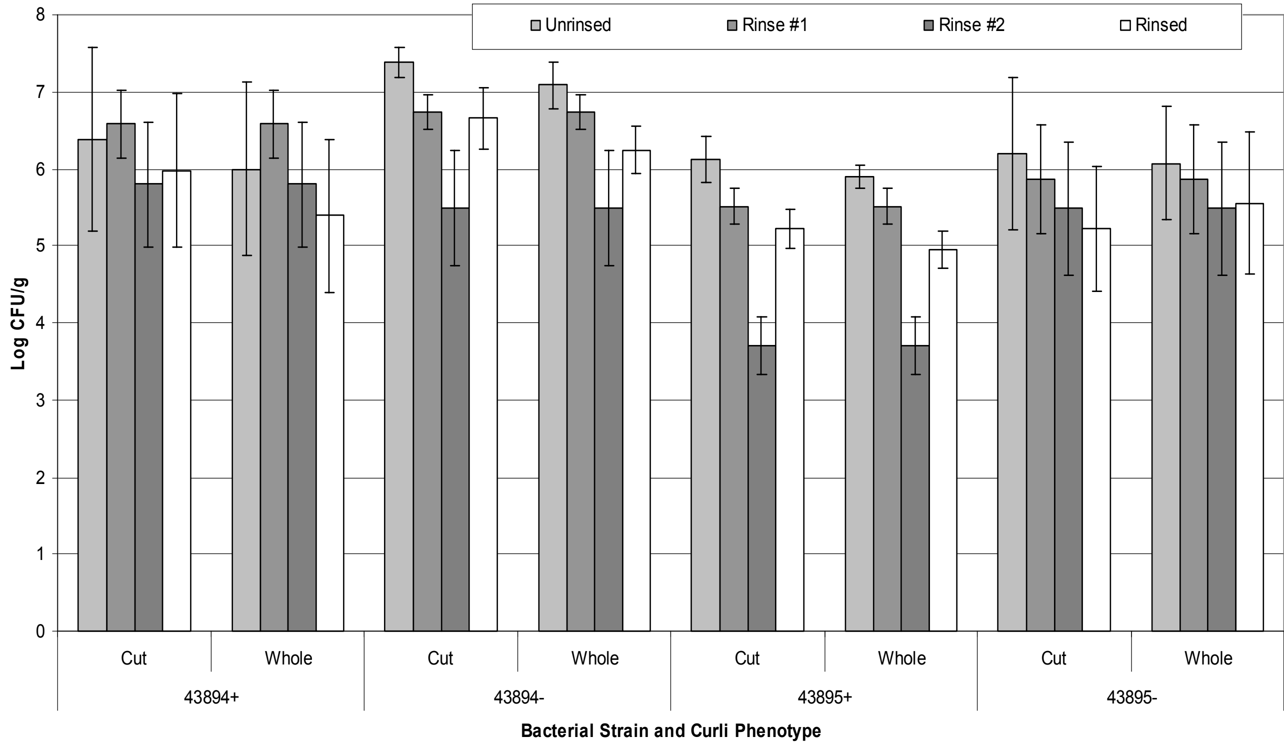


Figure 3.2 Attachment (Log CFU/g) of *E. coli* O157:H7 curli producing (+) and curli non-producing (-) strains (43894, 43895) to unrinsed and rinsed cut edge and whole leaf lettuce sections before and after two successive 1 min rinses (n=6). Unrinsed: concentration of cells attached to lettuce surfaces following inoculation. Rinse #1, 2: Concentration of cells left in the rinse wash following rinses. Rinsed: concentration of cells attached to lettuce surfaces after the two rinses.



CHAPTER #4

Role of *Escherichia coli* O157:H7 O-Side Chain on Cell Hydrophobicity, Charge and Attachment to Lettuce Surfaces.

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Abstract:

Escherichia coli O157:H7 foodborne outbreaks have been associated with lettuce. A better understanding bacterial attachment to produce surfaces could be helpful, advancing development of pre or post harvest interventions. Environmental factors during growing and harvesting of produce can contribute to contamination. Limited nutrients and extended periods of time in water may cause *E. coli* O157:H7 to shed its O antigen. Absence of the O157-polysaccharide antigen from the *E. coli* O157:H7 cell may influence bacterial attachment and affect the physicochemical properties (hydrophobicity, cell charge) of the cell. In this study, three strains of *E. coli* O157:H7 were used: 86-24 (wild-type), F12 (a mutant lacking the O-antigen) and pRFBE (plasmid containing O157 gene reintroduced). Strains were surveyed for hydrophobicity and cell charge using hydrophobic interaction chromatography and electrostatic interaction chromatography. Iceberg lettuce squares (2 x 2 cm) were inoculated with *E. coli* O157:H7 (~7 log CFU/square), dried in a laminar flow hood and rinsed twice with sterile de-ionized water. Strips (2 mm wide) of each cut edge of the lettuce were aseptically removed. Cut-edge and whole-leaf samples were homogenized and spiral plated onto Luria-Bertani Agar, supplemented with nalidixic acid (50 ppm), to assess levels of bacteria that remained on the lettuce leaf after rinsing. Neither the presence of O-polysaccharide nor leaf surface (cut vs. whole) influenced *E. coli* O157:H7 attachment to lettuce ($p>0.05$). However,

cells lacking the O antigen (strain F12) were significantly more hydrophobic than the wild-type or pRFBE strains ($p<0.01$). Surface charge differed among the strains tested ($p<0.01$); however, it did not influence bacterial attachment to lettuce surfaces. Results indicate that hydrophobicity and cell charge differences among strains of *E. coli* O157:H7 do not influence attachment to iceberg lettuce surfaces.

Introduction

Escherichia coli O157:H7 was identified as a foodborne pathogen in 1982 when it was connected to a case of hemorrhagic colitis. Classically, *E. coli* O157:H7 has primarily been a pathogen linked to foodborne outbreaks associated with ground beef. Over the past decade, however, it has been increasingly implicated in produce foodborne outbreaks. Between 1995 and 1996 alone, there were five outbreaks of *E. coli* O157:H7 where the vehicle of infection was lettuce *(53)*. Lettuce is the most common vegetable associated with *E. coli* O157:H7 produce outbreaks (34%) *(42)*. Other produce items associated with outbreaks include sprouts, cantaloupe and apple cider.

Bacterial attachment is thought to be the first step in the contamination of food products. *Escherichia coli* O157:H7 readily attaches to various produce surfaces and its removal has proven difficult *(8, 21, 24, 51, 55-58, 62, 67)*. Food safety research continues to evaluate the use of sanitizers and other rinses to remove pathogens from produce surfaces. While this approach is essential in the future of food safety, an understanding behind attachment mechanisms is crucial. A more complete understanding behind bacterial attachment may aid in preventing pre-post harvest contamination as well as the development of effective sanitizers and rinse agents.

Multiple elements may be associated with the attachment of *Escherichia coli* O157:H7 to produce surfaces. The mechanisms responsible for attachment of bacteria to various surfaces are poorly understood. It is hypothesized that attachment may be related to various specific factors (lipopolysaccharides, extracellular appendages, and various other outer membrane proteins) and/or non-specific factors (hydrophobicity and cell charge). Studies have evaluated the effect of fimbriae, outer membrane proteins,

hydrophobicity and cell charge on the attachment of bacteria to a variety of surfaces. Researchers have been unable to single out one mechanism associated with attachment.

Lipopolysaccharides (LPS) are thought to be a key mediator in the adhesion of bacteria to plant surfaces. LPS expression is involved in the attachment of *Rhizobium* spp. (plant symbiont), to the roots of legumes. It appears that LPS is involved in complex molecular communication between *Rhizobium* spp. and the plant host *(27)*

The lipopolysaccharide (LPS) surface of Gram negative bacteria consists of three parts: lipid A, the core oligosaccharide and the O-polysaccharide or O-antigen *(22, 23)*. Lipid A is embedded in the outer membrane and gives the cell hydrophobic characteristics. The core oligosaccharide, links lipid A to the O-polysaccharide (O-antigen) chain *(71)* . The core oligosaccharide consists of 2-keto-3-deoxyoctonic acid (KDO), and other sugar groups. The composition of the O-antigen is strain specific. *Escherichia coli* O157:H7 O-antigen is made up of four sugars (in this order):[*N*-acetyl-D-perosamine, L-fucose, D-glucose, and *N*-acetyl-D-galactose] *(71)*. Bilge et al. *(4)* identified the *rfbE* gene as being responsible for production of the O-antigen. The *rfb* gene cluster consists of 12-14 genes and is responsible for producing the enzymes necessary for the synthesis of the O157 antigen in *E. coli* O157:H7 *(4, 60)*.

The absence of the O-antigen increases adhesion of bacterial cells to animal cell lines remarkably *(4, 10, 63)*. Research has indicated that the bacterium's LPS can be altered by environmental conditions *(20, 25, 70)* Wang and Doyle *(70)* found that changes in the outer membrane take place in *E. coli* O157:H7 after surviving in water for extended periods *(70)*. *E. coli* O157:H7 survived in water for 21 months, and remained pathogenic and spontaneously lost its O-antigen after extended periods in water at 4 and

18°C *(20)*. This indicates that the loss of the O-antigen may occur under environmentally adverse conditions over an extended period of time, prior to contact of the bacteria with the produce surfaces. Adverse environmental conditions in the field (temperature, irrigation water) or processing plants (sanitation, desiccation) may influence attachment of the cells to food surfaces.

Alterations in the lipopolysaccharide layer of *E. coli* O157:H7 could affect physicochemical characteristics, such as hydrophobicity and cell charge. The hydrophobic lipid A embedded in the outer membrane, gives the cell its hydrophobic properties. The O-antigen is predominantly hydrophilic and can mask the hydrophobic properties of lipid A. In the absence of the O-antigen, cells are significantly more hydrophobic *(37, 69, 72)*. A cell's hydrophobicity may be an important factor associated with it's attachment to produce surfaces. Increased cell hydrophobicity can increase the ability of a cell to adhere to abiotic (stainless steel, polystyrene, and rubber) and biotic surfaces (soybean leaves, beef fat, meat, cantaloupe) *(3, 6, 14, 17, 28, 38, 67, 68)*. However, research is conflicting, and some researchers have found that the hydrophobicity does not affect attachment to apple, lettuce, and beef muscle *(14, 21)*.

Less is known about the influence of cell charge on attachment. Gram negative bacteria are widely known to have an overall negative cell charge. A negative charge is correlated with increased attachment of bacteria to stainless steel and beef muscle *(6, 14)*. However other research has shown that cell charge has no effect on attachment to stainless steel, beef, apples and lettuce *(17, 28, 68)*. Continued research in this area may provide a better understanding of bacterial attachment.