IDENTIFICATION OF PUTATIVE VIRULENCE FACTORS FROM *CAMPYLOBACTER* spp. ISOLATED IN ICELAND

By

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(Under the Direction of Mark Harrison)

ABSTRACT

Two studies were conducted to determine putative virulence factors of *Campylobacter* spp. that were isolated in Iceland. The first study investigated capacitance monitoring using a simplified medium for efficient and reproducible construction of growth curves for *Campylobacter* spp., which can be a time consuming and labor intensive process. When invasion assays are performed, it is required that *Campylobacter* spp. isolates be grown to a density of 10^6 to 10^8 CFU/ml. This investigation optimized conditions for use with the Bactometer® such that the determination of growth curves was achieved in a simple medium. Results suggested that isolates should be grown on Mueller Hinton plates under a microaerobic atmosphere (37°C; 24 h), then transferred to Mueller Hinton biphasic cultures for 6 h (37°C; microaerobic atmosphere). Serial dilutions should be used for inoculation of Bactometer® wells containing 1 mL Mueller Hinton broth plus 0.1M sodium pyruvate for obtaining growth curves. In the second study, putative virulence factors of *Campylobacter* spp. were investigated. *Campylobacter* spp. exhibited a wide distribution of adhesion and invasion ability, which was determined to be unrelated to *flaA* short variable region allele type. The second part of this study investigated the most invasive isolate 14118, the least invasive isolate, 13262, and two in

between to further understanding of the molecular basis of genetic diversity among these 4 *C. jejuni* isolates. DNA-DNA microarray hybridizations identified genes absent relative to *C. jejuni* 11168 (PMSRU). Several absent genes were located in 1 of 7 previously described plasticity regions. There were 372 genes determined to be present in *C. jejuni* isolates 14118, 5116, 8557 and 13262 as well as *C. jejuni* 11168 (PMSRU). DNA suppressive subtractive hybridizations identified genes not in common with *C. jejuni* 11168 (PMSRU). *C. jejuni* 14118 contained a gene from *C. doylei* 269.97 that encoded for a motility accessory factor. *C. jejuni* 13262 contained a cytolethal distending toxin operon from *C. lari.* as well as a type II restriction modification enzyme unlike isolate 14118, 5116 and 8557 which includes a type I restriction modification enzyme.

INDEX WORDS: *Campylobacter*, virulence, capacitance, Bactometer, cell invasion, microarray hybridization, suppressive subtractive hybridization

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DEDICATION

This dissertation is dedicated to

My family

For their prayers, constant support, and unconditional love

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CHAPTER 1

INTRODUCTION

Campylobacter spp. are curved rod, gram-negative bacteria with polar flagella (2). Campylobacteriosis is one of the most common bacterial intestinal disorders of humans in many industrialized countries (5). Human campylobacteriosis generally presents itself in 3-5 days after exposure with watery or bloody diarrhea, abdominal pain and nausea. Although normally self-limiting, other systemic infections can occur such as Guillain-Barre Syndrome and Reiter's Syndrome (6).

The consumption of poultry is considered the most likely route of infection. Broilers frequently carry large numbers of *Campylobacter* spp. in their intestinal contents. This carriage is asymptomatic and spillage of gut contents during processing can contaminate the retail poultry products and the abattoir environment (1). In the United States, retail chicken carcasses have estimated contamination rates of 60-80% with *Campylobacter* spp. populations averaging 10^6 for fresh chicken and 10^4 for frozen carcasses (3).

Reducing and eliminating foodborne human pathogens associated with poultry has received attention in the last years. The number of poultry being commercially processed grew from approximately 7.3 to 8.4 billion from 1994-2001, a 15% increase. Due to the increase of production and consumption of poultry, the national goal in the U.S, set by governmental agencies, is to reduce the incidence of *Campylobacter* infection from 13.37 per 100,000 people in 2002 to 12.30 per 100,000 people in 2010 (4). The Center for Disease Control and Prevention (CDC) reported that *Campylobacter* infection decreased 31% between 1996-1998. Since this decrease the estimated incidence of *Campylobacter* has not changed significantly. In 2007 the number of *Campylobacter* cases and incidence per 100,000 population was 12.79 (4). Since the Healthy People 2010 national health target has not been reached, there needs to be improved

understanding of the transmission of *Campylobacter* spp. from potential sources to human illness.

This dissertation used *Campylobacter* spp. isolates recovered during a comprehensive epidemiologic investigation conducted in Iceland. The original objectives were to investigate the ultimate sources and risk factors for transmission of *Campylobacter* spp. broiler flocks and their relevance to human exposure, utilizing multi-disciplinary international expertise. The study centered on the sampling of an entire broiler production population, which provides the sole source of broiler meat to the human population in Iceland. The multidisciplinary analysis of the results provide a perspective to describe sources and risk factors for exposure and infection of broilers, which demonstrate the greatest potential for reducing *Campylobacter* prevalence and concentration in poultry. It has also directed research efforts toward intervention technologies for reducing *Campylobacter* exposure in poultry and humans.

Iceland was targeted for this study due to its comparability to North American broiler production. All broiler chicken production in Iceland are derived from hatching eggs imported from grandparent breeder flocks in Sweden. Parent breeder chicks are distributed to three vertically integrated production lines, each with its own broiler hatchery. Based on visits and reviews of all on farm production, slaughter and processing, similarities to North American production are more remarkable than the differences. Poultry house construction though is more similar to Canadian poultry houses while the equipment and technology is consistent with both the U.S. and Canada. Production in Iceland is on a smaller scale with flock sizes ranging from <1,000 to 13,500. The limited scale of production and the fact that no broiler meat products are imported into Iceland, enabled a total population based epidemiological study. To compliment the closed system, molecular typing was employed and access to all human disease isolates and

available human case data was given. It would not be feasible for such an all inclusive population study in the U.S. due to scale of production, complex market distribution and disperse consuming population present numerous confounding factors, which can limit the inferential value of epidemiologic studies.

The purpose of this research is to better understand the molecular basis and biological consequences of genetic diversity among *Campylobacter* spp. for disease control. *Campylobacter* spp. isolates, recovered from poultry only and from poultry and humans, were surveyed to determine if "markers" might exist to allow us to determine if a *Campylobacter* isolate is possibly more likely to infect or cause disease in humans. The results from these experiments could lead to further investigations which may aid in better understanding of *Campylobacter* virulence and potentially lead to developing mitigation strategies to reduce the amount of human campylobacteriosis.

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CHAPTER 2

LITERATURE REVIEW

Campylobacter History and Characteristics

In 1886, Theodore Escherich observed stool samples of children with diarrhea that had organisms he believed resembled *Campylobacter*. In 1913, McFaydean and Stockman identified campylobacters, at the time called related *Vibrio*, in fetal tissues of aborted sheep. In 1957, King described *Campylobacter* from blood samples of children with diarrhea, and in 1972, Belgian clinical microbiologists first isolated *Campylobacter* from stool samples from patients with diarrhea. Due to the development in the 1970s of selective growth media more laboratories were able to test stool specimens for *Campylobacter* spp. Soon, *Campylobacter* spp. were established as common human pathogens (6) and are recognized as the leading cause of bacterial foodborne diarrheal disease throughout the developed world (46).

The Family *Campylobacteraceae*

This family is comprised of gram negative, nonsaccharolytic bacteria with microaerobic growth requirements (3-5% carbon dioxide, 3-15% oxygen) (8, 62) and have a small genome of approximately 1.6-1.7 Mbp of AT-rich DNA; the GC ratio is approximately 30% (35). Members of the family occur primarily as commensals or parasites in humans and domestic animals. Cells in the family *Campylobacteraceae* are curved, S-shaped, or spiral rods that are 0.2 to 0.8 μm wide, 0.5 to 5 μm long and non-sporeforming (48). Cells in old cultures may form spherical or coccoid bodies. They are typically motile with a characteristic corkscrew motion by means of a single polar unsheathed flagellum at one or both ends of the cell. Cells grow under microaerobic conditions (42) and have a respiratory and chemoorganotrophic type of metabolism. However, some species of *Campylobacter*, such as *C. sputorum*, *C. concisus*, *C. mucosalis*, *C. curvus*, *C. rectus*, and *C. hyointestinalis* require an atmosphere containing an increased concentration of hydrogen to be isolated (42) . Enzymes such as superoxide dismutase (SOD), catalase,

peroxidase, glutathione synthetase, and glutathione reductase in *C. jejuni* are believed to play an important role in providing oxygen toxicity protection. *Campylobacter* spp. are fastidious organisms that require complex growth media (61). Energy is obtained from amino acids or tricarboxylic acid cycle intermediates, not carbohydrates (62).

Campylobacter spp. are referred to as thermophilic *Campylobacters* and grow best at 37 to 42°C, with an optimal temperature of 42°C, which reflects the adaptation to the intestines of birds (29, 64). Typical biochemical characteristics are reduction of fumarate to succinate; negative methyl red reaction and acetoin and indole production; and for most species, reduction of nitrate, absence of hippurate hydrolysis, and presence of oxidase activity (62).

Clinical Features

Campylobacter enteritis is considered to be a foodborne disease, with infection often being derived from a range of foods and also water-based environmental sources (35). It has been reported that every year in the United States there are about 20-150 cases per 100,000 people. There is a high isolation rate among young adults (15-24 years old), approximately 8 per 100,000 per year. Under-reporting is significant and the true incidence could be five to ten times higher (38). The vast majority of cases appear to be sporadic; unlike with *Salmonella* and *Escherichia coli*, outbreaks of campylobacteriosis are rarely observed (6). Sporadic illnesses peak during summer months and are associated with mishandling or consumption of undercooked poultry or cross- contamination of other foods by raw poultry (7). Outbreaks of *Campylobacter* spp. have resulted from cross-contaminated chicken, raw milk, and untreated water (6, 41).

Under certain conditions, for example on exposure to atmospheric oxygen, bacteria can become spherical or coccoid in shape. This change in shape has been associated from a viable

culturable form to a viable but not cultural form (VNC) and was first proposed by Colwell et al. following a study on survival of *Salmonella* in aquatic systems (53). It has been suggested that this VNC state acts as an adaptation for survival in adverse environments such as low nutrient availability or upon entry into stationary phase (41). Rollins and Colwell were the first to report a VNC form of *C. jejuni* and since then there has been debate as to whether the form for *Campylobacter* truly exists (52). There has been evidence that shows that VNC *Campylobacter* spp. are infectious in neonatal mice but in a chicken model the evidence is more contradictory. Such investigations are difficult to perform and interpret because not all coccoid cells may progress to a VNC state or VNC development may advance through several stages in a coccoid cell (35).

Human campylobacteriosis generally presents as 3-5 days of acute watery or bloody diarrhea, usually with severe abdominal pain, fever and general malaise (15). Most cases of infection are due to *C. jejuni*, with only 10% due to *C. coli* and less than 1% *C. lari* (38). In a volunteer study, *C. jejuni* infection occurred after ingestion of as few as 800 organisms (6). In another volunteer study, inoculations with large doses (10^5 to 10^8 colony forming units) were required for infection. Most infections are due to exposure to relatively low numbers of organisms that must multiply in the host to achieve a clinically apparent outcome. Examination of colonic biopsies shows an acute inflammatory response with infiltration of the epithelium and lamina propria with neutrophils and mononuclear cells. Among infected persons in developed countries, both leukocytes and erythrocytes are nearly always present in stools, indicating the universality of the inflammatory process, even when stools are watery and not grossly bloody. Thus, *Campylobacter* colitis or enteritis must be considered an inflammatory disease (65).

Although campylobacterosis is generally self-limiting, incapacity may last several weeks and up to 10% of reported cases may require medical intervention.

More serious systemic infections are well recognized and occur most commonly in the immnocompromised. Chronic sequellae, like arthropathies, are not uncommon and an associated of campylobacteriosis with postinfectious neuropathies such as Guillain-Barrè syndrome have been identified (38). Guillain- Barrè syndrome is an acute inflammatory demyelinating neuropathy that occurs from a cross reaction with Schwann-cells or myelin. It results in neuromuscular paralysis. An estimated one case of GBS occurs for every 1,000 cases of campylobacteriosis (5). *Campylobacter* spp. are also associated with Reiter syndrome, a reactive arthropathy that affects the ankles, knees, wrists, and the small joints of the hands and feet. The duration of arthritis ranges from several weeks to several months. Although the arthritis can be incapacitating, full recovery is generally seen. The pathogenesis of GBS and Reiter syndrome is not completely understood (6).

Treatment for campylobacteriosis usually involves rehydration, but antimicrobial therapy may be required for patients who have high fever, bloody diarrhea, or more than 8 stools in 24 h; immunosuppressed patients, patients with bloodstream infections, and those whose symptoms worsen or persist for more than 1 week from the time of diagnosis (6). Erythromycin is the drug of choice, but ciprofloxacin is now frequently used in adults (38) and fluoroquinolones and tetracyclines are used as alteratives (15).

Pathogensis

Many pathogen-specific virulence determinants may contribute to the pathogenesis of *C. jejuni* infection in humans, but none has a proven role. Potential determinants of pathogenicity include chemotaxis, motility, and flagella, which are required for attachment and colonization of

the gut epithelium (6). *Campylobacter* enters the host intestine by way of the stomach acid barrier and colonize the distal ileum and colon. Following colonization of the mucus and adhesion to intestinal cell surfaces, *Campylobacter* perturbs the normal absorptive capacity of the intestine by damaging epithelial cell function either directly, by cell invasion or the production of toxin(s), or indirectly following the initiation of an inflammatory response. These possibilities are not mutually exclusive; any combination may have a role depending on the host status and attributes of the infecting strain (35).

The ability of *C. jejuni* to colonize the human gastrointestinal tract is essential for disease. Binding to epithelial cells prevents the colonizing bacteria from being swept away by mechanical cleansing forces such as peristalsis and fluid flow. Binding is prerequisite for entry into host cells. The ability of *C. jejuni* to invade cultured cells appears to be strain dependent (36). Recent advances include the identification and characterization of virulence factors required for *C. jejuni* binding, entry, and survival within host cells.

Chemotaxis and Motility

Effective colonization requires chemotaxis. *Campylobacter* spp. have mechanisms to detect chemical gradients and linked motility functions that enable the cell to move up or down the gradient. Non-chemotactic mutants failed to colonize the suckling mouse intestine. Studies have revealed various chemoattractants including mucin, L-serine and L-fucose, while several bile acids have chemorepellant effects. Motility of *Campylobacter* spp. necessitates the production of the flagellum which is an important virulence factor (35). Functional flagella presumably help the bacteria overcome the clearing movement of peristalis and enable them to enter and cross the mucus layer overlaying the epithelium (65). They are able to move through viscous environments at speeds up to 75 μm/s (18). *C. jejuni* contains one or two polar flagella

that cause the typical darting motility observed by microscopy and the moist appearance of colonies on agar plates. The flagellar filament consists of multimers of the protein flagellin and is attached by the hook protein to a basal structure, which is embedded in the membrane and serves as a motor for rotation (65). The flagella are complex and are composed of two related subunits, FlaA and FlaB (37). They are >93% homologous to each other and are encoded by genes which are adjacent on the chromosome but regulated by distinct promoters. The *flaA* gene is regulated by the flagellar promoter σ^{28} and the *flaB* gene is regulated by a σ^{54} promoter (27). Early studies with genetically undefined mutants indicated that the flagellum was needed for adhesion and for colonization in a range of animals (35). Mutation of *flaA* resulted in the synthesis of a truncated flagellar filament, composed of *flaB*. This flagellar filament greatly reduced motility compared to that of the wild type flagellum. Mutation of *flaB* resulted in a flagellar filament which was indistinguishable from the wild type filament in length and with motility that was reduced slightly compared to the wild type (27).

Adhesion and Invasion

C. jejuni is increasingly associated with episodes of gastrointestinal illness in developed countries. Although little is known about the pathogenic events required for illness, *C. jejuni* strains differ in their ability to adhere to and invade intestinal epithelial cells and it has been suggested that this is important to the pathogenic process (51). Grant et al. found that flagella are not involved in *C. jejuni* adherence to epithelial cells but that they do play a role in internalization (25). Another study by Konkel et al. showed that not only was either the FlaA or FlaB gene required for invasion but that *Campylobacter* invasion antigens (Cia proteins) were also needed for maximal invasion. This data also showed that the *C. jejuni* Cia proteins are secreted from the flagellar export apparatus (37). Hanel et al. showed that there was correlation

between invasion of Caco-2 cells (human colon cells) and colonization in the chick gut. Isolates that did not colonize the chick gut were not invasive with the Caco-2 cells. Strains that showed to be strong colonizers had high invasion rates with the Caco-2 cells (28). Monteville et al. (40) published that the CadF outer membrane protein appears to promote the binding of *C. jejuni* to fibernectin, thereby stimulating the host cell signaling events associated with bacterial uptake. Fibronectin is a glycoprotein that is present at regions of cell to cell contact in the gastrointestinal epithelium, providing a potential binding site for pathogens (40). Zheng et al. (68) and Datta et al. (22) reported for an isolate to be invasive it needs the *flaA* gene, *cad,* and other genes such as *ciaB*, *pldA*. Genes of the pVir plasmid are also involved in host cell invasion. The genes *cdtA*, *cdtB*, and *cdtC* are responsible for the expression of *Campylobacter* cytolethal distending toxin, which induces the proinflammatory cytokine production of epithelial cells and causes host cell cycle arrest, cell distention and eventually cell death (68).

Poultry Production

In humans, *Campylobacter* infections are primarily the result of the ingestion of contaminated foods of animal origin such as poultry (46, 55). In poultry, *Campylobacter* is a commensal oraganism and colonizes the mucus overlying the epithelial cells primarily in the ceaca and small intestine, but may be recovered from other places in the gastrointestinal tract and from the liver and spleen.

Campylobacter contamination of poultry flocks, horizontal transmission and/or vertical transmission, remain unclear. Horizontal sources that could be vectors of infection include environment of the poultry house, hatchery pads, litter, feed, water, personnel, small animals on the farm, flies and rodents (21, 34, 55). However, none of these suspected sources has been identified conclusively as the formal source of infection on broiler farms. In many cases there is

no comparison of isolates from broilers and the environment by phenotypic and genotypic typing methods, leading to significance of these putative sources of infection. *C. jejuni* was probably detected in suspect sources after the broilers had come infected, suggesting that broilers, instead of being infected from environmental sources, might be the source of environmental contamination. It is very difficult, in many situations, to determine which contamination came first (55).

C. jejuni is very sensitive to oxygen and drying so the organism is generally unable to grow in feed, litter or water under normal ambient conditions (32). *Campylobacter* has been isolated from water lines and reservoirs of bird houses, and these strains may be phenotypically and genotypically identical to what is found in feces. However the water contamination usually follows rather than precedes the colonization of a flock (44).

Flies, mice and other pests can act as a vector for the transmission of *C. jejuni (31)*. Identical serotypes and genotypes have been isolated from both broilers and insects within broiler houses; however the direction of spread has not been determined (55). Stern et al. (58) found 25% of insects caught outside of poultry houses to be *Campylobacter* positive four weeks prior to detection of *Campylobacter* in flocks. The bacteria though, can only survive on or within these insects for a few days (44).

Campylobacter has also been shown to be present and possibly transferred by workers as well as equipment. The organism has been isolated from footbath water, farmer's boots and transport crates (58). Therefore it is reasonable to believe transmission of *C. jejuni* may be spread between flocks and houses by personnel. Nesbit et al. (43) showed two adjacent broiler houses that lacked biosecurity measures were colonized with different genotypes of *C. jejuni* even though the two houses shared equipment and the same farmer worked both houses.

Vertical transmission has been debated as a theory on the mode of transmission. Sahin et al. were not able to recover *Campylobacter* from eggs that had been inoculated with the bacterium. They sampled for the presence of *C. jejuni* in eggs placed in an incubator to hatch and in newly hatched chicks (54). In another study though, Van de Giessen and others have shown vertical transmission as a means of contamination of a breeder flock. *C. jejuni* isolates from a parent flock were found to be from the same clonal origin as those from the offspring in the broiler flock (34). *Campylobacter* has also been isolated from various segments of the reproductive tract of the chicken (16). *C. jejuni* can be recovered from the oviduct, which suggests a possibility of egg contamination and it also has been found in semen samples from breeder cockerels (31).

Poultry Processing

When a flock of broiler chickens becomes positive for *Campylobacter*, the prevalence of infection is high, often reaching 100% of the birds tested (26). The high number of *Campylobacter* positive birds can still be found in the first stages of processing. In a poultry processing plant, there are typically six basic functions: pre-scalding, scalding, defeathering, evisceration, washing, and chilling. Poultry are eviscerated, and as the skin is not normally removed, many contaminants are found on the skin (20). Son et al. (57) reported that *Campylobacter* was isolated from 78.5% of the carcasses sampled from three sample sites (pre-scald, pre-chill, and post-chill). The pre-scald and pre-chilled sites had the highest *Campylobacter* contamination at 92% and 100%, respectively (57). Berrang and Dickens (12) found that *Campylobacter* was the highest when sampled pre-scald (4.7 $\log_{10}$) and the counts dropped significantly after the carcasses were scalded (1.8 $\log_{10}$). When the carcasses exited the chill tank, the incidence of *Campylobacter* was almost the same as what was recorded post-scald.

The potential for cross-contamination during scalding could occur due to the follicles remaining open throughout the processing until the carcess is chilled. Once the follicles close during chilling, the organisms may become trapped (60). Contamination levels could be directly related to bacterial levels in the processing water. Water samples from both bird washers and chlorinated chiller water have been found *Campylobacter* positive (10).

Several mitigation steps have been incorporated in poultry processing to help control *Campylobacter* contamination. Chlorine has been used for more than 40 years in poultry processing to reduce spoilage bacteria, control the spread of pathogens, and prevent buildup of microorganisms on surfaces and equipment (34). Berrang et al. (11) reported that application of chlorine in the chill tank was significantly related to a larger reduction in *Campylobacter* numbers (P=0.0003). However, the difference with the overall reduction was small. Oyarzabal et al. (45) found that the post-chill application of acidified sodium chlorite to chicken carcasses caused a significant reduction in *Campylobacter* numbers. Rapid freezing of carcasses offers additional control measures. An Icelandic study suggested that frozen poultry poses a lower risk to health than fresh meat (59). A method used in European countries that will kill *Campylobacter* and other infectious bacteria is the use of irradiation using electron beams or high energy electromagnetic radiation. Studies have shown that *Campylobacter* are more susceptible to radiation than *Salmonella* and *Listeria monocytogenes* (31).

Other mitigation steps focus on preventing contamination from fecal or cecal sources. One study discovered the odds of having a positive skin sample were 35 times greater when the same carcasses had *Campylobacter* positive intestines (33). Berrang et al. (13) tested the effect of placing vinegar in the cloaca prior to defeathering, which is a processing step that has been

known to increase *Campylobacter* spp. levels. They found the use of vinegar resulted in a significantly lesser increase of *Campylobacter* levels.

Despite mitigation steps, as much as 98% (17) of retail broilers have been reported as being positive for *Campylobacter* spp. and levels of contamination may vary between 10^2 and 10^5 CFU per carcass (46). It is likely that many consumers purchase poultry products that are contaminated. How consumers handle products at home will ultimately affect the cross-contamination and infection rate.

Microarray Technology and Suppression Subtractive Hybridization

A wealth of nucleotide sequence data from the genomes of bacterial pathogens has become readily available. Comparative analysis of bacterial genomes provides information on the physiology and evolution of bacteria and allows for detailed comparisons between related bacteria. The availability of whole genome sequences has led to the development of microarray and suppression subtractive hybridization technology. These two methods provide researchers tools to continue the investigation into why some bacterial strains are more virulent than others.

Microarrays

Microarrray technology has rapidly advanced and gained in popularity over the years. A microarray comprises a large number of genes deposited onto a glass slide, which are used for a mutiplex reaction, essentially a large dot blot (66). The identification of genes present in a cellular sample as well as genes expressed by hybridizing cDNA from reverse transcribed RNA can be determined through microarrays. It should be noted, however, that microarrays are limited to pre-determined genes of interest, which are printed on a slide. Also, some genes may be expressed and are regulated at the translational or post-translational level, so this type of expression would not be detected by microarrays.

Microarrays began in the mid-1990s with the use of glass microscope slides for printing DNA (56). Glass is a good choice as a microarray substrate due to its low fluorescence, low cost, high heat resistance, and rigidity. Slides must be cleaned and coated for DNA immobilization/spotting to occur on glass (1). Glass can be coated either adsorptively, as with poly-l-lysine, or covalently, as with functionalized silanes. Functionalized silanes are most commonly used since they can bind directly to nucleic acids (56). The polyanionic DNA interacts with the polycationic surface by way of coulombic attraction. After printing of the slide, the DNA is locked to the surface by ultraviolet irradiation or baking. Although non-covalent mechanisms are typically used, covalent binding has advantages. The DNA can be oriented in a certain way to increase probe availability for hydridization, allow less non-specific binding, allow for the use of more stringent washes, and offers a potential for stripping and re-hybridizing arrays (9). An example of covalent binding is the use of amino modified DNA to bind to epoxide activated glass slides. Amino alcohols form when the epoxides react through nucleophillic displacement (1).

Fluorescently labeled cDNA is hybridized to slides using either direct or indirect labeling. Direct labeling of cDNA is accomplished by a reverse transcriptase reaction with mRNA as a template, dye conjugated nucleotides, and oligo (59)n as primers (14). Indirect labeling incorporates amino-allyl dUTP into the cDNA and the primary amine groups are subsequently conjugated to succinimidyl ester of Cy3. This labeling method increases labeling density, however, it is also known to decrease the intensity of fluorescent signal (49).

Microarray experiments must be replicated since chips can fail or be distorted. If a study is to find large differences, then a design with 3 samples per group is adequate (50). After data is

collected, it must be normalized to remove technical differences. Local regression is typically used for normalizing microarrays (24).

Microarray Experiments on *Campylobacter*

Pearson et al. (47) investigated diversity in 18 *C. jejuni* strains from diverse sources using microarrays. In total, 16.3% of the genes present in the sequenced strain NCTC11168 were either absent or highly variable in sequence among the strains of *C. jejuni* examined in this study. Seven major plasticity regions (PR) were also identified in the genome and they comprised 50% of the variable gene pool. PR 1 contained genes important in the utilization of alternative electron acceptors for respiration and may confer a selective advantage to strains in restricted oxygen environments. PR 2, 3, and 7 contain many outer membrane and periplasmic proteins and hypothethical ones of unknown function. PR 4, 5, and 6 contain genes involved in the production of surface structures including LOS, flagellum, and post-translational glycosylation of the flagellum. These variable regions identified in this study highlight genetic factors that might be linked to phenotypic variation and adaptation to different ecological niches. Another study used oligonucleotide microarrays for a rapid and accurate simultaneous differentiation among *C. jejuni*, *C. coli*, *C. lari*, and *C. upsaliensis*. The array contained species-specific oligonucleotide probes developed by using specific regions of five genes (*fur*, *gly*A, *ceu*B-C, and *fli*Y) (63). Champion et al. (19) used microarrays to examine 111 *C. jejuni* strains isolated from humans, chickens, bovine, ovine, and the environment. NCTC 11168 was used as the control in the experiment. The *C. jejuni* functional core was determined by calculating the number of genes that were present in each of the test strains as well as the control strain. The species-specific functional core consisted of 979 predicted coding sequences comprising 59.2% of the genome and was involved with regulatory, metabolic, cellular, and biosynthetic processes.

DNA microarray analysis has also been used to determine if isolates from patients with Guillain-Barré syndrome (GBS) differ from isolates from patients with uncomplicated gastrointestinal infection (39). Microarray analysis did not identify discrete groups of isolates or any unique features within the genome of the *C. jejuni* isolates associated with GBS. It could not be determined if the lack of hybridization in various regions represents the absence of a particular gene or nucleotide divergence within an existing gene. Additionally, differences due to the presence of genetic elements in either the GBS or enteritis related isolates would not necessarily be detected because of the absence of such elements in the genome of the strain used to construct the microarray.

Suppresion subtractive hybridization

Suppresion subtractive hybridization is a technique designed to identify those regions present in one genome but absent from another (2). This method was first reported in 1996 and applied to the study of *Helicobacter pylori* (4). Genomic DNA extracted from a driver strain is hybridized with DNA extracted from a tester strain with a view to isolating those sequences that are present in the tester strain but absent from the driver strain (23). To facilitate the process, the driver strain DNA must be present in excess. It is important to ensure that tester DNA sequences are short so a digestion by restriction enzymes before hybridization can occur. The tester DNA is then separated into two portions, each of which is subjected to a ligation reaction to attach a different adaptor sequence to the 5' ends. The two portions are then separately hybridized to the driver DNA (in excess). All sequences that hybridize with the driver DNA should be mopped up leaving only tester specific single-stranded sequences. When the two tester portions are mixed and hybridized together, only those sequences unique to the tester strain will have different adaptors present on each strand. PCR is then used to detect these sequences. Only the sequences

that carry both adaptors, one on each end, will amplify. The PCR products are cloned into a vector to produce a subtracted library. Although this procedure is not entirely effective, >50% of clones should be tester-specific (67).

Supression subtraction hybridization experiments

Hepworth et al. (30) looked at the use of suppression subtractive hybridization to extend our knowledge of *C. jejuni* genome diversity. They carried out five subtractions between *C. jejuni* isolates from different sources such as rabbit, cattle and wild birds. They wanted to determine the variability within and between common multilocus sequence type (MLST) clonal complexes. The results of the study showed a correlation between clonal complex and the distribution of the metabolic genes. There was, however, no evidence to support the hypothesis that host preference may have any role in the distribution of such genes. Another study by Ahmed et al. (3) researched genetic differences between two *C. jejuni* strains with different colonization potentials. *C. jejuni* NCTC 11168 has been shown in preliminary studies to be a poor colonizer whereas strain 81116 is a better colonizer in chickens. The technique of subtractive hybridization was used to identify gene fragments of strain 81116 not present in strain 11168. Six clones were found with similarities to restriction-modification enzymes found in other bacteria. Two inserts had similarity to arsenic-resistant genes, and four others had similarities to cytochrome c oxidase III, dTDP-glucose 4,6-dehydratase and an abortive phage-resistance protein. Some of these genes may be involved in colonization potential.

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Chapter 3

Simplified Capacitance Monitoring for the Determination of *Campylobacter* spp. Growth Rates

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ABSTRACT

Capacitance monitoring is commonly used as an efficient means to measure growth curves of bacterial pathogens. However, the use of capacitance monitoring with *Campylobacter* spp. was previously determined to be difficult due to the complexity of the required media. We investigated capacitance monitoring using a simplified medium for the efficient and reproducible construction of growth curves for *Campylobacter* spp. Initially *Campylobacter* spp. were grown on Mueller Hinton plates under a microaerobic atmosphere (37°C; 24 h), followed by transfer to Mueller Hinton biphasic for 6 h (37°C; microareobic atmosphere). Serial dilutions were used for inoculation of Bactometer® wells containing 1 mL Mueller Hinton broth plus 0.1M sodium pyruvate for the completion of *Campylobacter* spp. growth curves with the Bactometer®.

INTRODUCTION

Campylobacteriosis is one of the most common bacterial intestinal disorders of humans in many industrialized countries (Knudsen et al., 2006). There are approximately 20-150 cases per 100,000 reported foodborne illness cases per year in the United States. However, under-reporting is significant and the true incidence could be as high as five to 10 times the reported rate (Lee and Newell, 2006). Epithelial cell invasion is considered to be an essential step in *Campylobacter* spp. infection. Invasion studies using intestinal epithelial cells as well as other cell lines show that the relative ability to invade cultured cells is strain dependent (Poly et al., 2004). When invasion studies are performed, it is required that *Campylobacter* spp. isolates be grown within a range of 10^6 to 10^8 CFU/ml. Consequently, determination of growth curves for *Campylobacter* spp. can be a time consuming and labor intensive process prior to completing cell invasion or other assays such as gene content or transcriptome analyses using microarrys for this organism.

Microbiological conductimetric methods are often used to monitor bacterial growth. Conductimetric instruments monitor microbial metabolism within a growth medium by the measurement of significant changes in electrical activity including total impedance, conductance, or capacitance (Corry et al., 1995; Wawerla et al., 1999; Line and Pearson, 2003). The Bactometer® microbial monitoring system (bioMerieux, Hazelwood, MO) is one such conductimetric instrument that was previously determined to be efficient for monitoring growth patterns for other bacterial types such as *Salomonella* spp. and *Escherichia coli* (Wawerla et al., 1999). Each Bactometer® module contains 16 wells that contain approximately two mL total volume each, with two electrodes exposed in each well. Due to strict media and atmospheric requirements for growth of *Campylobacter* spp., the use of capacitance monitoring with this

organism was determined to be either labor intensive (requiring complex media formulations) or inefficient and irreproducible with less complex media formulations (personal communications, Patricia Rule). In this investigation, we optimized conditions for use with the Bactometer® such that the efficient and reproducible monitoring of *Campylobacter* spp. for determination of growth curves was achieved in a simple medium.

MATERIALS AND METHODS

Ten *Campylobacter* spp. isolates (Table 3.1), originally recovered from Iceland, were used for this investigation (Stern et al., 2003). *Campylobacter* spp. require a microaerobic atmosphere (3-5% oxygen, 2-10% carbon dioxide, with a balance of nitrogen) for optimal growth (Altekruse and Swerdlow, 2002). As the Bactometer® does not allow for the manipulation of atmosphere, a 0.5 ml overlay of sterile mineral oil was used in each well to help reduce oxygen form entering wells . Conditions used for optimization are listed in Table 3.2. For the first trial, *Campylobacter* spp. isolates were grown for 24 h on Mueller Hinton agar plates at 37°C under microaerobic conditions followed by transfer to biphasic cultures (25 mL vented capped T-flask containing a 10 mL Mueller Hinton agar base and 5 mL Mueller Hinton broth) for an additional 18 h under the same conditions (Rollins et al., 1983). Serial dilutions, ranging from 10^{-1}-10^{-6} were performed, with 100 μL of each dilution added to individual Bactometer® wells containing 1 mL of Mueller Hinton Broth, followed by the addition of mineral oil. Modules were placed into the Bactometer® at 37°C for 48 h following the manufacturer's instructions. Each trial was replicated in triplicate, but no growth was obtained under these initial conditions. Trial 2 was similar with the exception that half of the wells contained 0.2 mL of Mueller Hinton agar to mimic the biphasic culture. After 48 h, no growth was detected in any of the Bactometer® wells, thus biphasic conditions in the wells were not

tested in subsequent experiments. A third trial included the use of 20 mM sodium pyruvate in the primary biphasic cultures during the 18 h of growth. Sodium pyruvate is an organic salt involved with amino acid metabolism and initiates the Kreb's cycle where glucose is converted to energy such as ATP. It has also been shown to have protective effects against reactive oxygen species (Corry et al., 1995). Serial dilutions were once again placed into the Bactometer® wells containing 1 mL of Mueller Hinton broth overlayed with a 0.5 mL of sterile mineral oil; again there was no growth of isolates in the Bactometer®. In the fourth trial, the molar concentration of sodium pyruvate was increased to 0.1 M. These conditions resulted in bacterial growth at 48 h, however, the initial bacterial numbers exceeded the Bactometer® threshold value of 10^6 CFU/mL. For the fifth trial, the *Campylobacter* spp. isolates were grown in primary biphasic cultures containing a 0.1M concentration of sodium pyruvate, for only 6 h. The Bactometer® wells, containing 1 mL Mueller Hinton broth supplemented with 0.1M sodium pyruvate, were inoculated in triplicate with serial dilutions of the *Campylobacter* spp. isolates. After incubation in the Bactometer® for 48 h, growth curves were successfully obtained as reported in Figure 3.1. A final trial was conducted to determine if 0.1M sodium pyruvate was beneficial when added to both the primary biphasic cultures as well as the Bactometer® wells which only contained Mueller Hinton broth.

RESULTS AND DISCUSSION

A protocol was developed after all seven trials were completed. Ten *Campylobacter* spp. isolates were grown on Mueller Hinton agar plates for 24 h at 37°C and then inoculated into primary biphasic cultures containing Mueller Hinton broth for an additional 6 h of growth at 37°C under microaerobic conditions. Serial dilutions, 10^{-1}-10^{-3}, were prepared and 100 μL of each dilution was placed into wells that contained a 0.1M concentration of sodium pyruvate

along with 1 mL of Mueller Hinton broth. A 0.5 mL overlay of sterile mineral oil was used to help create the microaerobic conditions. All isolates were inoculated in triplicate. Growth curves and threshold times were obtained after incubation in the Bactometer® for 48 h at 37°C (Figure 3.1, Table 3.1).

Our results demonstrate that growth rate can vary greatly among *Campylobacter* isolates, therefore, this method will be useful in studies where establishment of growth curves is critical for subsequent experimental analyses of these bacteria. Our recommendation is that initial *Campylobacter* spp. be grown on Mueller Hinton plates under a microaerobic atmosphere (37°C; 24 h), followed by transfer to Mueller Hinton biphasic cultures for 6 h (37°C; microaerobic atmosphere). Serial dilutions (10^{-1}-10^{-3}) should then be used for inoculation of Bactometer® wells containing 1 mL Mueller Hinton broth supplemented with 0.1M sodium pyruvate, followed by an overlay with 0.5 mL of sterile mineral oil.

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Table 3.1: Time required for different strains of *Campylobacter* spp. to reach a detection limit of approximately 10^6 CFU/mL by capacitance measurement using the Bactometer®.

Isolate	Source	Threshold Time (h)
81-176	human isolate	12
13262	broiler ceca	12
14590	other poultry	12
14131	human isolate	7
14194	poultry production environment	6
4568	domestic species	6
7358	parent breeder fecal	6
12826	broiler ceca	6
8889	parent breeder fecal	6
5069	broiler ceca	6

Table 3.2: Experimental *Campylobacter* spp. growth conditions evaluated for detection of changes in capacitance using the Bactometer®

Trials	Primary Biphasic Cultures			Bactometer Well Medium					Growth
	18 h	6 h + Sodium Pyruvate	6 h	Mueller Hinton		Sodium Pyruvate		Mineral Oil	
				1.0 mL Broth	0.2 mL Agar	20 mM	0.1 M	0.5 mL	
1	x			x				x	No
2	x			x	x			x	No
3	x			x		x		x	No
4	x			x			x	x	Yes
5		x		x			x	x	Yes
6		x	x	x			x	x	Yes
7			x	x			x	x	Yes

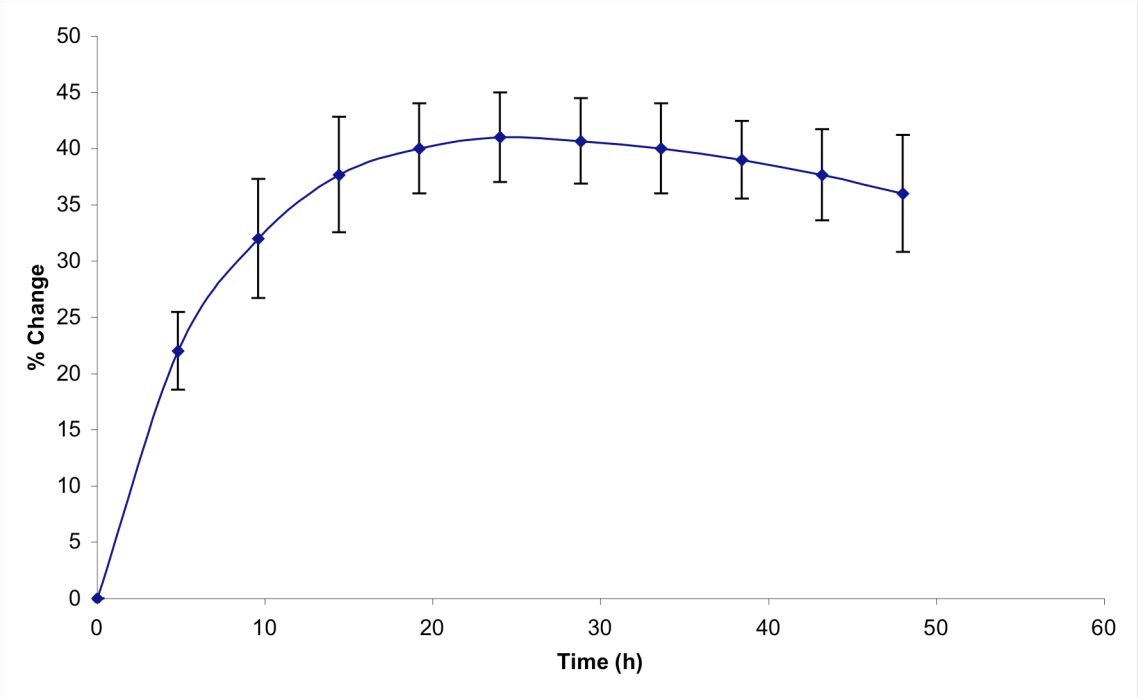

Figure 3.1: Typical mean capacitance response for *Campylobacter* spp. 4568 inoculated into Bactometer® wells at 10^1 CFU/mL. Bars represents standard deviation.

Chapter 4

Identification of Putative Virulence Factors Utilizing Invasion Assays and Whole Genome Comparisons of *Campylobacter* spp. Isolates Recovered from Iceland

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Abstract

Background

Campylobacter spp., one of the most common causes of human foodborne bacterial gastroenteritis, is commonly associated with the handling and consumption of raw poultry products and considered to be a major source of *Campylobacter* induced disease in humans. The United States has sought to reduce the number of cases of campylobacteriosis in humans through investigations of the epidemiology and pathogenicity of *Campylobacter* spp. These investigations should be facilitated by employing a library of epidemiologically related *Campylobacter* spp. isolates. The isolates used in this investigation were originally recovered from an extensive epidemiologic investigation conducted in Iceland. We investigated the adherence and invasiveness of 52 *Campylobacter* spp isolates using human colon cells as well as the genetic diversity of four *C. jejuni* isolates that demonstrated a wide range of invasiveness towards human colonic cells.

Results

Campylobacter spp. exhibited a wide range of adhesion and invasion ability, which was determined unrelated to *flaA* short variable region (SVR) allele type. Four isolates comprised of the most invasive isolate (14118), the least invasive (13262), and two in between were selected for comparative genomic analysis. DNA:DNA microarray hybridizations identified genes absent relative to 11168 (PMSRU). Several genes were located in 1 of 7 previously described plasticity regions. There were 372 genes determined present in *C. jejuni* isolates 14118, 5116, 8557, and 13262 as well as *C. jejuni* 11168 (PMSRU). Suppressive subtractive hybridizations identified genes absent from *C. jejuni* 11168 (PMSRU). *C. jejuni* 14118 contained a gene from *C. doylei* 269.97 that encoded for a motility accessory factor and a gene involving transport. *C. jejuni*

13262 contained a cytolethal distending toxin (CDT) operon from *C. lari* as well as a type II restriction modification enzyme unlike isolates 14118, 5116 and 8557 which includes a type I restriction modification enzyme.

Conclusions

These studies provide further insight into genetic variability of *Campylobacter* spp. The results facilitate the determination of the core *C. jejuni* genome and also provide information regarding putative virulence factors that might explain differences in adhesion and invasiveness. Further investigations of variably present genes, such as hypothetical proteins, should lead way to more knowledge for the development of intervention strategies and biomarkers.

Background

Infection due to *Campylobacter* spp. exposure to a variety of foods, water, and environmental sources is one of the major causes of human diarrheal disease in industrialized countries [1, 2]. Although *Campylobacter* spp. can asymptomatically colonize the intestinal tract of a variety of warm blooded animals, this pathogen often results in human disease ranging from self-limiting gastroenteritis to more serious systemic infections [3]. The majority of *Campylobacter jejuni* cases are enteric, with episodes confined to local acute gastroenteritis characterized by nausea, abdominal pain, diarrhea, and fatigue. Although campylobacteriosis is generally self-limiting, incapacity may last several weeks and up to 10% of cases require medical intervention [4]. *Campylobacter* spp. infections have also been associated with extra-intestinal sequalia such as Guillain-Barré Syndrome [5] and reactive arthritis [6].

The reduction and elimnation of the occurrence of foodborne pathogens associated with poultry has received attention in recent years. The increase of commercially processed poultry, which grew from approximately 7.3 to 8.4 billion from 1994-2001 [7], and the subsequent consumption of poultry, the national goal in the U.S., set by governmental agencies, is to reduce the incidence of *Campylobacter* spp. infection from 13.37 per 100,000 people in 2002 to 12.30 per 100,000 people in 2010 [8]. The Center for Disease Control and Prevention (CDC) reported that *Campylobacter* spp. infection decreased 31% between 1996-1998 likely due to the implementation of a new regulation known as FSIS Pathogen Reduction/HACCP Regulation [9]. Since this decrease, the estimated incidence of *Campylobacter* spp. has not changed significantly. In 2007 the number of cases and incidence per 100,000 population were 12.79 for *Campylobacter* spp.[10]. Since the Healthy People 2010 national health target has not been

reached, a need exists to improve understanding of the epidemiology and transmission of *Campylobacter* spp.

Poly et al. [11] found that the ability of different *C. jejuni* isolates to invade cell cultures is strain dependent. Noninvasive strains have been isolated from patients with noninflammatory disease, while invasive strains were isolated from patients with inflammatory diarrhea. These findings suggest that different *Campylobacter* spp. isolates vary in their virulence properties and that these virulence properties are correlated at least in part, with the ability to invade human intestinal epithelial cells [11]. The percent invasion tends to vary based on cell line and specific isolate. Hickey et al. [12] reported that *C. jejuni* 81-176 invaded INT407 cells at a level of 2.1% whereas other campylobacters invaded INT407 cells within a range of 0.001%-0.41%. Hanel et al. [13] investigated 11 *C. jejuni* isolates and determined the range of invasiveness of Caco-2 cells was between 0.00003%-2.14%. The ability of pathogenic bacteria to adhere to host tissues is important for the establishment of an in vivo niche. This binding can be a prerequisite for host cell invasion as with *Campylobacter* spp. [14]. An emerging theme among pathogenic bacteria is their ability to utilize host cell molecules during the infectious process to facilitate their binding and entry into host cells [15].

Investigations into the basis of *Campylobacter* spp. transmission could be facilitated by a library of epidemiologically related *Campylobacter* spp. isolates. A comprehensive epidemiologic investigation was previously conducted in Iceland because the small size allowed for sampling of a well-defined broiler production and processing industry. Iceland's broiler practice is comparable to that of North America and provides the sole source of broiler meat to the Icelandic human population. Additionally, production is on a smaller scale with flock sizes ranging from <1,000 to 13,500 [16]. The limited scale of production and the fact that no broiler

meat products are imported into Iceland enabled an unique total population based epidemiological study that would not be feasible in the U.S. due to the scale of production, complex market distribution, and disperse consuming population.

The epidemiologic analysis included all poultry flocks, other agriculturally important animals, environmental sources, and human clinical cases over a three-year period. *flagellinA* short variable region (*flaA* SVR) DNA sequence analysis of recovered isolates revealed that certain *flaA* SVR subtypes were recovered from chickens as well as from humans. However, there were also *flaA* SVR subtypes that were predominate in poultry, but never recovered from humans. This observation allowed for investigations to facilitate our understanding of the molecular basis and biological consequences of genetic diversity of *C. jejuni* and human disease [17]. Additionally, this information is important to determine if "biomarkers" might exist to allow us to determine if a *Campylobacter* spp. isolate is more likely to result in human disease. To address these goals, cell adhesion/invasion assays, suppressive subtractive hybridizations, and DNA:DNA microarray analyses were utilized to determine genetic differences between invasive and noninvasive *Campylobacter* spp. isolates.

Materials and Methods

Bacterial Isolates and Growth Conditions

Fifty-two *Campylobacter* spp. isolates, with known spatial and temporal relationships, were used for this investigation (Table 4.1) [16]. These isolates were previously determined to segregate into one of four unique *flaA* SVR allele types. Allele types were selected after comparing the genetic distances between all isolates; the four allele types demonstrating greatest distance were selected (Figure 1). *C. jejuni* 81-176 and *C. jejuni* 11168 (PMSRU) were also included in this study as a control for adhesion/invasion assays, suppressive subtractive

hybridizations, and DNA:DNA microarray analyses respectively. All *Campylobacter* spp. isolates were grown at either 42°C or 37°C for 24 h on Meuller-Hinton (MH) agar (Sigma, St. Louis, MO) under microareobic conditions (5% O_2, 10% CO_2, and 85% N_2). Isolates were then grown in Mueller-Hinton biphasic cultures for 16 h at 37°C under microareobic conditions to reach a mid-log phase. *Escherichia coli* DH5α mcr- and TOP10 (Invitrogen, Carlsbad, CA) cells were cultured aerobically at 37°C on Luria-Bertani (LB) agar plates for 24 h. Transformed TOP10 *E. coli* were grown at 37°C in LB broth supplemented with 50 μg/ml of ampicillin.

DNA Isolation

For DNA:DNA microarray hybridization and suppressive subtractive hybridization analyses, *Campylobacter* spp. isolates were grown to stationary phase as previously described [18]. Genomic DNA was isolated using a phenol-chloroform extraction. Breifly, cells were pelleted, lysed using an SDS extraction buffer, RNAse and proteinase K treated followed by precipitation with 0.2 volume of 10M ammonium acetate. Plasmid DNA was isolate using the Qiagen Plasmid MiniPrep Kit (Valencia, CA), from overnight cultures of transformed *E. coli* TOP10 cells.

Cell Culture

Caco-2 cells (provided by Dr. Holly Sellers, PDRC, University of Georgia) were cultured as cell monolayers in modified Eagle medium (MEM) (Cellgro, Herdon, VA) supplemented with nonessential amino acids (Cellgro), sodium pyruvate (Cellgro) and 20% fetal bovine serum (FBS) (Sigma) and incubated at 37°C in a 5% CO_2 humidified incubator [19, 20]. For experimental assays, Caco-2 cell monolayers were seeded at a density of approximately 1 x 10^5 cells into 24 well plates. The plates were incubated at 37°C in a 5% CO_2 humidified incubator

for 16 h prior to infection to allow reformation of the monolayer [21]. Prior to the assay, the cell monolayers were washed with phosphate-buffered saline (PBS) pH 7.2 (Invitrogen) [13].

Adherence and Invasion Assay

The bacteria, *Campylobacter* spp. and *E. coli* DH5α mcr-, were harvested from Mueller Hinton biphasic cultures incubated at 37°C in microaerobic for 12 h and aerobic atmospheres for 12 h, respectively. One mL of cell suspension was removed and centrifuged at 4,000 x rpm for 10 min. Each pellet was resuspended in 1.5 ml of MEM + 1% FBS. The assay was performed by co-incubating mid-log phase isolates in triplicate with semi-confluent Caco-2 cells. Each resuspended isolate (0.5 mL) was inoculated into duplicate wells. Serial dilutions (10^1- 10^7) were prepared where 100µl was inoculated onto plates to determine the number of bacteria inoculated into each well [13]. MH agar, incubated at 37°C in a microareobic atmosphere for 36 h, was utilized for *Campylobacter* spp. isolates while LB agar, incubated at 37°C for 24 h was used for *E. coli*. All plate counts were performed in duplicate. After inoculation, the Caco-2 cells were incubated for 3 h at 37°C in a 5% CO_2 humidified incubator to allow bacterial adhesion and internalization. For determination of adherence, one-half of the cells were washed three times with sterile PBS and the cell monolayer lysed with 0.1% Triton X-100 (Sigma) [14, 22]. The total bacteria associated with the cells (intracellular and extracellular bacteria) were enumerated by plating serial dilutions (10^1-10^4), in duplicate on either MH or LB agar. The MH agar plates were incubated at 37°C in a microaerobic atmosphere for 36 h prior to counting. LB agar plates were incubated at 37°C in ambient atmosphere, for 24 h before counting. The remaining wells were measured for bacterial invasion. The infected cells were washed twice with sterile PBS and incubated in fresh culture medium containing 250 µg per ml of gentamicin (Sigma) for 3 h to kill remaining viable extracellular bacteria [20]. Quantification of the viable

intracellular bacteria was performed by washing the infected Caco-2 cells with sterile PBS twice followed by subsequent lysis with 0.1% Triton-X 100 [14, 22]. Serial dilutions, (10^1-10^3) were plated in duplicate on their respective agars and incubated accordingly as described above.

flaA SVR DNA Sequence Analysis

flaA SVR DNA sequence analysis was performed as previously described [23]. Isolated colonies of *Campylobacter* spp. were suspended in 300 µl of sterile H_2O and placed at 100°C for 10 min. Ten µl of each boiled cell suspension was used as a template for *flaA* SVR PCR with the following primers: FLA242FU: 5' CTA TGG ATG AGC AAT TWA AAA T 3' and FLA625RU: 5'CAA GWC CTG TTC CWA CTG AAG 3'. A 35 cycle reaction was used with 1 min denaturing at 96°C, 1 min annealing at 52°C and 1 min extension at 72°C. The resulting product was approximately 425 bp. Sequence data was generated using either the FLA242 FU primer or the FLA625 RU primer with the Big Dye Dye-Terminator Cycle Sequencing Kit (ABI-PE, Foster City, CA). Data was assembled and edited using Sequencer 4.7 (Genes Codes Corp., Ann Arbor, MI) and aligned using Clustal X (Plate-Forme de Bio-Informatique, France). Aligned sequences were compared and dendrograms were generated using the Neighbor-Joining algorithm with HKY85 distance measurements in PAUP*4.0 [24].

Campylobacter spp. Speciation

Campylobacter spp. were speciated using a multiplex PCR as previously described by Wang et al. [25].

Suppressive Subtractive Hybridization

Suppressive subtractive hybridization was performed [26] using the PCR-select bacterial genome subtraction kit (Clonetech, Palo Alto, CA) with modifications as described below. *C. jejuni* isolate 11168 (PMSRU) was used as the driver while four *C. jejuni* isolates (5116, 14118,

13262, and 8557) recovered from Iceland were used individually as tester samples. Four micrograms of genomic DNA from each isolate was digested with 30 units of AluI (New England Biolabs, Ipswich, MA) and 30 units of DraI (New England Biolabs, Ipswich, MA) for 30 min to produce blunt end fragments ranging approximately 100 to 650 bp in size [17, 27]. Both the first and second subtractions were performed at 59°C. Thermal cycling conditions for enrichment of subtracted sequences were altered to 72°C for 5 min followed by 30 cycles at 94°C for 30 s, 64°C for 30 s and 72°C for 1.5 min.

Preparation of the Subtractive-Hybridization Library

Products resulting from PCR amplification were ligated in pCR®2.1 vector using the TA Cloning® Kit Verson V (Invitrogen, Carlsbad, CA) at 14°C. The ligated products were transformed into One Shot TOP10 chemically competent *E. coli* cells using the TA Cloning® Kit Verson V and tranformants were selected for kanamycin and ampicillin resistance. White colonies were picked, transferred to LB broth supplemented with ampicillin (50 μg/ml), and grown at 37°C overnight with agitation (200 rpm). Plasmid DNA was isolated as previously stated.

DNA Sequence Analysis

Plasmid DNA was digested with 40 units of EcoRI (New England Biolabs) and resolved in a 0.8% agarose gel. Plasmids that released an insert upon digestion were further analyzed by DNA sequence analysis, using the Big Dye Terminator v3.1 Sequencing kit (PE Applied Biosystems, Foster City, CA). DNA sequences were assembled and edited using Sequencher 4.7 (Gene Codes Corporation; Ann Arbor, MI), and similarity searches performed using BLASTN and BLASTX (http://www.ncbi.nlm.nih.gov/BLAST/)[28]. Unique DNA sequences obtained

during this investigation were submitted to GenBank (accession numbers provided upon final revision).

Microarray Construction, DNA Labeling, DNA:DNA Microarray Hybridization, and Analysis

DNA microarrays used for analysis were prepared as previously described [27]. Primers from the BioPrime Labeling Kit (Invitrogen) were used for random primer labeling of genomic DNA in the presence of amino-allyl dUTP (Ambion, Austin, TX), followed by coupling to either Cy3 or Cy5 (GE Healthcare, Piscataway, NJ) monoreactive flours [18]. Microarrays were hybridized overnight at 42°C and subsequently visualized using a Packard Scan Array Light 2-color scanner with Scan Array Express Software Version 1.1 (Packard BioScience, La Jolla, CA). Three independent hybridization experiments were performed yielding 9 measurements per gene (given that each gene is present thrice on each microarray). Analyses were preformed using the software program GACK (genomotyping analysis; C.Kim [Stanford University, Stanford, CA]; available at http://cmgm.stanford.edu/falkow/whatwedo/software/software.html). This program calculates an idealized normal distribution curve for each array and assigns a binary value to each data point on the microarray based on an estimated probability that a gene is present or absent in a given isolate, relative to 11168 (PMSRU), the genome sequenced isolate.

Results

Campylobacter spp. *flaA* SVR Allele Types

Four *Campylobacter* spp. *flaA* SVR allele groups were chosen for analysis based on comparison of the genetic distances between all *Campylobacter* spp. isolates from the original Iceland investigation (Table 4.1, Figure 4.1). The first *flaA* SVR allele type, DAA, contained 21 isolates ranging in sample origin from human clinical isolates, other poultry isolates (turkey, etc), domestic species, socks (environmental samples), broiler caeca samples, parent breeder fecal

samples, retail product samples, and grandparent fecal samples. *flaA* SVR allele type DAB was comprised of 15 isolates which also included human clinical isolates, broiler caeca samples, other poultry samples, parent breeder fecal samples, and retail product samples. The third *flaA* SVR allele type, DAC, included 5 isolates that were collected from broiler caeca samples, wild bird samples, parent breeder and rearing fecal samples. The last *flaA* SVR allele type, DAD, included 11 isolates from domestic species samples, human clinical samples, other poultry samples, parent breeder fecal samples and retail product samples. Interestingly human clinical isolates were found only in three *flaA* SVR allele types with no human isolates originating from DAC *flaA* SVR allele type.

Adhesion of *Campylobacter* spp. with Caco-2 cells

Fifty-two *Campylobacter* spp. isolates (Table 4.1) were used in cell adhesion assays with *E. coli* DH5α mcr- employed as the negative control, and the highly invasive *C. jejuni* 81-176 [29] employed as the positive control. The average percent adhesion ranged from 0.00008% to 3.4%. *C. jejuni* 11168 (PMSRU) adhered at 0.025% and *C. jejuni* 81-176 adhered at 0.083%. A histogram illustrating the distribution of *flaA* SVR allele groups relative to percent adhesion is presented in Figure 4.2. The level of adhesion was arbitrarily assigned to 3 groups; low (<0.08%), medium (0.08-0.8%), and high (>0.8%). Each *flaA* SVR allele type was represented in each adhesion level. Sixty-six percent of *flaA* SVR allele group DAB was located in the low adhesion range. *flaA* SVR allele group DAD isolates (n=7) were found primarily in the medium range whereas at the high level, allele type DAC had 40% of its isolates (2 out of 5).

There appeared to be no relationship demonstrated between host of recovery source and level of adhesion as illustrated in Figure 4.3. Four of nine human isolates adhered at a percentage of 0.71% or greater. Of the remaining 5 human isolates, 4 fell within the middle

adherence range of 0.1%-0.051% while one *C. jejuni* isolate was present in the low range with an adherence of 0.01%. The poultry isolates were distributed throughout all levels of adhesion.

Invasion of *Campylobacter* spp. within Caco-2 cells

Isolates were also tested for their invasiveness of a human Caco-2 cell line. The percent invasion ranged from 0.000003% to 1.2%. *C. jejuni* 11168 (PMSRU) invaded at 0.035% whereas *C. jejuni* 81-176 invaded at 0.75%. Again, percent invasion was arbitrarily divided into 3 levels; low (<0.0025%), medium (0.0025-0.01%), and high (>0.01%) (Figure 4.4). In the high range of invasion, *flaA* SVR allele group DAA occurred the most frequently followed by *flaA* SVR allele groups DAB and DAD. The most invasive isolate, belonging to *flaA* SVR allele DAD, invaded at a higher level than did *C. jejuni* 81-176, the positive control. Interestingly, *flaA* SVR allele group DAC (n=5), which contained no human isolates, was not found in the high invasive range. All five of the *flaA* SVR allele group DAC isolates invaded Caco-2 cells below 0.002%. These isolates were recovered from such sources as parent breeder fecal samples, wild birds, parent rearing fecal samples, and commercial broiler caeca. Isolates belonging to *flaA* SVR allele group DAB, recovered from broiler caeca, retail products, human clinical samples, parent breeder fecal samples, other poultry fecal samples, and parent rearing fecal samples, were found between in all levels of invasion. Four of fifteen isolates (26.6%) within this *flaA* SVR allele group were found to be highly invasive whereas 11 of fifteen isolates (73.3%) were found to have a percent invasion less than 0.0025%. No isolates fell into the medium range of invasiveness with this particular allele group.

Figure 4.5 represents the distribution of isolate source relative to percent invasion. The majority (7 of 9) of the human isolates collected invaded the Caco-2 cells at a rate greater than 0.003%. Four out of the nine human isolates demonstrated invasion levels greater than 0.012%

while the four remaining human isolates invaded within the range of 0.002% to 0.01%. Only one human isolate was placed in the low invasive range at 0.001%. The isolates recovered from wild birds and other domestic animals were not found to be invasive, with invasion rates of Caco-2 cells at <0.0006%. The other poultry isolates were distributed within the three invasion ranges.

DNA:DNA Microarray Hybridization Analysis

Four *Campylobacter* spp. isolates, chosen on the basis of the invasivness of Caco-2 cells, were used for subsequent DNA:DNA microarray hybridization assays. Isolate 14118 demostrated the highest percent invasion (1.2%) to the Caco-2 cells, isolate 13262 demonstrated low percentage (0.00005%) of invasion while isolates 5116 and 8557 fell in the medium range of the invasion of Caco-2 cells. Isolate 14118 (high invasion) did not have 11 genes relative to *C. jejuni* 11168 (PMSRU) (Table 4.2) while isolate 5116 (medium invasion) did not have 18 genes (Table 4.3). The absent genes from isolate 14118 included 4 genes coding for hypothetical proteins with yet to be defined functions. The remainder of the absent genes coded for integral membrane proteins (*cj0860*), transferases (*cj0407* & *cj1331*), and transport proteins that link inner and outer membranes (*cj0753c*). Isolate 5116 (medium range) missing genes included a putative binding protein (*cj0412*), a bacterioferritin (*cj1534c*), involved in oxidative damage protection, integral membrane proteins, multidrug transporter membrane component (*cj1587c*) and a chemotaxis signal transduction protein (*cj0262c*). There were also six hypothetical proteins with unknown function determined absent. Hybridization analyses demonstrated that of these four isolates, isolate 8557 (medium invasion range) demonstrated the greatest similarity to *C. jejuni* 11168 (PMSRU). There were only three genes of the 1634 tested from the11168 (PMSRU) genome that were identified as absent in 8557 (Table 4.4). The three genes represent a

hypothetical protein (*cj0056c*), a putative aminotransferase (*cj1436c*) and a *parB* family protein (*cj0101*), which is a predicted transcriptional regulator. The least invasive isolate, 13262 was determined to be the most divergent with 66 genes absent relative to 11168 (PMSRU) (Table 4.5). These genes are related to virulence properties and included cell division proteins, integral membrane proteins, the cytolethal distending toxin, binding proteins and 19 hypothetical proteins.

There were 372 genes determined to be conserved between *C. jejuni* isolate 11168 (PMSRU) and all four *C. jejuni* isolates 14118, 5116, 8557 and 13262 tested using DNA microarray analyses. The common sequences included the core genes responsible for metabolic, cellular, and regulatory processes (Table 4.6) [30]. Fifty-nine of the genes (15.9%) were determined to have unknown function.

Identification of Unique *Campylobacter* spp. DNA Fragments Present in *C. jejuni* isolates relative to *C. jejuni* 11168 (PMRSU) by Suppressive Subtractive Hybridization

Suppressive subtractive hybridizations were performed to identify DNA sequences present in *C. jejuni* isolates 14118, 8557, 5116, and 13262 that are absent in 11168 (PMSRU). The initial round of subtractive hybridization at 63°C indicated that the subtraction hybridization had occurred at a low efficiency. This has been recognized as a previous issue with *Campylobacter* spp. [17]. The hybridization temperature was thus lowered to 59°C, which greatly enhanced the annealing of homologous driver and tester DNA, and the subsequent amplification of tester-specific DNA. The number of subtracted clones for all four isolates along with the number of those clones determined to contain inserts and the clones determined to be false positive, is presented in Table 4.7.

Subtracted sequence analysis of isolate *C. jejuni* 14118 (high Caco-2 invasion level) resulted in 68 clones, 2 showing redundancy such that 66 unique clones were analyzed (Table 4.8). The unique *C. jejuni* 14118 clones differing from 11168 (PMSRU) were found to possess significant similarity to *Campylobacter* spp. other than *C. jejuni* 11168 (PMSRU) and the similarities are listed in Table 4.9. The unique subtracted clones that differ from *C. jejuni* demonstrated similarity to *C. doylei* 269.97 and *Blastopirellula marina*.

Subtracted sequence analysis of isolate *C. jejuni* 5116 (medium Caco-2 invasion level) resulted in 24 clones, 8 showing redundancy such that 16 unique clones were analyzed (Table 4.10). The unique clones determined to differ from 11168 (PMSRU), found to possess significant similarity to *Campylobacter* spp. other than *C. jejuni* 11168 (PMSRU), are listed in Table 4.11. Clones determined to differ from *C. jejuni* demonstrated similarity to *C. doylei* 269.97 and *Campylobacter* phage CGC-2007. One clone was also determined to be of hypothetical or unknown function.

Subtracted sequence analysis of isolate *C. jejuni* 8557 (medium Caco-2 invasion level) resulted in 79 clones, 8 showing redundancy such that 71 unique clones were analyzed (Table 4.12). The unique clones differing from 11168 (PMSRU) were found to possess similarity to *Campylobacter* spp. other than *C. jejuni* 11168 (PMSRU), are presented in Table 4.13. The unique clones that differed from *C. jejuni* demonstrated similarity to *C. doylei* 269.97 and *Campylobacter* phage. Nine subtracted clones were determined to be similar to either unknown or hypothetical proteins with unknown function.

Subtracted sequence analysis of isolate *C. jejuni* 13262 (low Caco-2 invasion level) resulted in 59 clones, 4 showing redundancy such that 55 unique clones were analyzed (Table 4.14). The clones determined to differ from 11168 (PMSRU) possessed significant similarity to

Campylobacter spp. other than *C. jejuni* 11168 (PMSRU) (Table 4.15). The unique clones that differed from *C. jejuni* were *C. lari*, *C. doylei* 269.97, and *Beggiatoa* spp. Eleven clones were determined to be either hypothetical proteins or have unknown function.

For all four *C. jejuni* isolates, the subtracted sequences grouped into 8 general functional categories as defined by Poly et al. [11]. The first functional group is cell envelope and surface structures while the second category was designated a restriction modification, recombination and repair category. The third category is transport and the fourth category is small molecule metabolism. Bacteriophage sequence comprises another category with the sixth category involving bacterial toxins and chemotaxis is the seventh category. The last category was hypothetical and unknown proteins. Figure 4.6 presents the number of analyzed clones within each functional category for all four *C. jejuni* isolates.

Discussion

It was predicted that cell invasion assays and whole genome comparison would provide insight as to whether certain *flaA* SVR allele types of *Campylobacter* spp. are more pathogenic to humans. The *flaA* short variable region (SVR) is a single locus that has been utilized as a reliable typing method [23, 31, 32]. This typing method has been found to be more discriminatory than serotyping or PCR-restriction fragment length polymorphism of the *flaA* gene. Investigations have demonstrated that *flaA* SVR comparisons can follow the spread of *Campylobacter* spp. populations within the poultry industry [33]. However, Dingle et al. [34] determined that since the *flaA* SVR region had a great allelic diversity at the nucleotide sequence level, this typing technique was not useful for long term population and evolutionary analyses. The *flaA* SVR typing technique is useful for discriminating between related isolates, for example, distinguishing outbreak strains [23]. Knudsen et al. [35] found that phenotypic and

genetic typing methods have proven useful for epidemiological studies of *Campylobacter* spp. infections; however, the prevalence of a specific genotype was not necessarily an indicator for colonizing ability. The results from the current study demonstrated that the *flaA* SVR was not indicative of cell invasiveness and thus there was no correlation between adherence/invasion and the *flaA* SVR allele type. Hanel et al. also reported no association between *flaA* type and invasion into Caco-2 cells when they investigated 11 *C. jejuni* isolates of different origin [13]. Other researchers have demonstrated that the ability to invade human cell lines and the degree to which *Campylobacter* spp. invades eukaryotic cells is dependent on the *Campylobacter* spp. strain. Ketley [36] also stated that clinical isolates appear to be more efficient in the invasion of human cells. This is not in agreement with the results of this investigation as isolates recovered from human clinical cases demonstrated invasion at both high and low levels. Biswas et al. [37] found that the ability to invade cultured cells is also strain dependent but quite variable in efficiency.

Studies show that *C. jejuni* translocation through invaded cells occurs due to a transcellular process rather than via intercellular spaces [38]. The specific function for translocation is not known, but it does depend on functional flagella [20]. Individual host cell type could also play a role with invasion of *Campylobacter* spp. into epithelial cells. The internalization mechanism triggered by *C. jejuni* has been associated with the combined effect of microfilaments (MF) and microtubules (MT) of host cells [22]. Most invasive bacteria including *Salmonella*, *Shigella*, *Listeria* and *Yersinia* spp. [21] trigger microfilament dependent entry pathways. *Neisseria* and *Klebsiella* spp. require both microfilaments and microtubules for invasion. *C. jejuni* has been shown to be less invasive by both MF and MT depolymerization with human intestinal cells [37]. Since the *flaA* SVR is not indicative of virulence, each isolate within an allele type may potentially differ in their genome type.

Whole genome comparison techniques including DNA:DNA microarray hybridization and suppressive subtractive hybridization, were utilized to further identify genetic differences in the four *C. jejuni* isolates that exhibited different cell invasion abilities. Pearson et al., using DNA:DNA microarray hybridizations, [39] characterized seven hypervariable plasticity regions, PR1-7, among *C. jejuni* isolates recovered from diverse origins. PR 1 contains genes encoding the molybdenum transport apparatus and pantothenate biosynthesis genes. PR 2 consists of putative membrane transporters and hypothetical proteins whereas PR 3 consists of ABC transporters and hypothetical proteins. PR 4 contains the N-acetyl neuraminic acid synthase genes, which are involved in the sialylation of lipooligosaccharide (LOS). The LOS biosynthesis genes and post-translational modification of the flagellin genes are located in PR 5. The last two regions code for capsule biosynthesis (PR 6) and membrane proteins (PR 7) [30, 39].

Isolate *C. jejuni* 14118, the most invasive isolate, revealed only one gene missing related to *C. jejuni* isolate within PR 5. This gene, *cj1331*, codes for *pmtB* acylneuraminate cytidylyltransferase for flagella modification. Isolate 8557 had one absent gene relative to *C. jejuni* 11168 (PMSRU) from PR 6. Gene *cj1436c* is a putative aminotransferase and is involved in capsular polysaccharide biosynthesis. *cj1724c* was absent relative to *C. jejuni* 11168 (PMSRU) from isolate 5116 and is within PR 7. This gene codes for a 7-cyno-7-deazaguanine reductase. The least invasive isolate, 13262, revealed absence of five genes relative to *C. jejuni* 11168 (PMSRU) within the seven plasticity regions. Gene *cj0303c*, which codes for *modA,* a putative molybdate-binding lipoprotein, belongs to PR 1. Gene *cj0487*, a hypothetical protein, was absent from isolate 13262 and this particular gene falls within the 2nd PR. The third PR includes genes from ABC transporters, membrane and hypothetical proteins. One gene, *cj0737*,

fell within this region. This gene is a putative periplasmic protein and is involved in heme utilization or adhesion. The least invasive isolate, *C. jejuni* 13262, also had two genes absent relative to *C. jejuni* 11168 within PR 6. This region includes genes from the capsular biosynthesis locus. Gene *cj1435c* is a hypothetical protein whereas *cj1436c* is a putative aminotransferase. Both these genes are involved with amino acid transport and metabolism for polysaccharide biosynthesis.

The use of suppressive subtractive hybridization [26] resulted in the recovery of gene sequences determined to be similar to several *Campylobacter* spp. other than *C. jejuni*. Isolates 5116 and 8557, the mid-range invasive isolates, contained clones demonstrating similarity to that of *Campylobacter* spp. phage genes. Previous investigations revealed that up to a total of 89% of the *C. jejuni* isolates and 14% of *C. coli* isolates could be infected by at least one of the bacteriophages [40]. Isolates 5116 and 8557 also had genes similar to those found in *C. doylei* 269.97. These genes are involved in capsular polysaccharide biosynthesis. *C. doylei* can cause both gastritis as well as enteritis and are isolated more often from blood cultures than stool cultures [41]. A five year study conducted in Australia reported that *C. doylei* was isolated from 85.2% of *Campylobacter*/*Helicobacter* related bacteremia cases [41].

The most invasive isolate, 14118, also contained a gene from *C. doylei* 269.97 that encoded for a motility accessory factor. Flagella-mediated motility is recognized to be one of the factors contributing to *C. jejuni* virulence. The motility accessory factor (maf) is a family of flagellin-associated proteins that is involved in flagella glycosylation. This large cluster also contains genes thought to be involved in sugar biosynthesis and transport [42]. An additional *C. doylei* gene involving transport was also found in isolate 14118. This invasive isolate contains a major facilitator superfamily (MSF) protein. MSF is a set of drug efflux proteins that can

contribute to both natural insensitivity to antibiotics and to emerging antibiotic resistance thus these may be potential targets for the development of new antibacterial drugs [43].

The least invasive isolate, 13262, contained a cytolethal distending toxin (CDT) operon from *C. lari*. As previously discussed, the microarray hybridization results determined that this isolate had the *cdtA* gene from *C. jejuni* 11168(PMSRU) absent. The pathogenicity of the species *C. lari*, generally isolated from the intestines of gulls, shellfish, fish and other animals is unknown [44]. Isolate 13262 also has a type II restriction modification enzyme unlike isolates 14118, 5116 and 8557 which include a type I restriction modification enzyme. The role of restriction modification systems in *Campylobacter* spp. is unclear. These specific enzymes might be involved in the breakdown of foreign DNA [45]. These enzymes might also be necessary for stimulating the formation of DNA fragmentation and recombination, resulting in antigenic diversity and variation, such as the homologous recombination observed for the virulence-associated flagellin locus of *C. jejuni* [46]. In a study with *Helicobacter pylori* the presence of restriction modification proteins was associated with the ability of the bacteria to infect its host [47]. This may suggest that these enzymes might affect virulence gene expression. *C. jejuni* could have a similar function where these enzymes control expression of genes involved in colonization.

Lastly, all isolates include multiple unknown and hypothetical proteins, which will require further research into the significance of their contribution to potential virulence. The identification of virulence genes and gene products could help improve diagnostic methods and help determine intervention strategies.

Conclusion

The determination of virulence factors is important for the reduction of campylobacteriosis in humans. Although the invasion assays did not show that *flaA* SVR subtype was an indicator of virulence, invasion assays did reveal a wide range of invasiveness. DNA:DNA microarray hybridization and suppressive subtractive hybridizations revealed genetic differences between the isolates that should facilitate our understanding of variability in virulence. Future studies should now focus on the putative virulence factors elucidated in this study along with the numerous hypothetical proteins with unknown function. Further knowledge of *Campylobacter* spp. virulence should lead to novel intervention strategies including vaccines that target invasive *Campylobacter* spp.

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Table 4.1. *flagellinA* short variable region (SVR) allele, sample number, and sample origin of *Campylobacter* spp. isolates employed in this investigation.

FlaA SVR Allele	Sample Number	Sample Origin
DAA	7845	Human Isolate
	14590	Other Poultry
	13759	Human Isolate
	14131	Human Isolate
	4568	Domestic Species
	14194	Socks
	16229	Other Poultry
	5069	Broiler Caeca
	12826	Broiler Caeca
	12578	Broiler Caeca
	8952	Parent Breeder Fecal
	7678	Parent Breeder Fecal
	7486	Parent Breeder Fecal
	7303	Parent Rearing Fecal
	7599	Parent Rearing Fecal
	11393	Parent Rearing Fecal
	12920	Retail Product
	13829	Retail Product
	12620	Retail Product
	10914	Grandparent Fecal
	10905	Grandparent Fecal
DAB	8557	Human Isolate
	8559	Human Isolate
	14078	Human Isolate
	5116	Broiler Caeca
	9801	Broiler Caeca
	14347	Other Poultry
	13719	Broiler Caeca
	11408	Parent Breeder Fecal

Table 4.1 cont. *flagellinA* short variable region (SVR) allele, sample number, and sample origin of *Campylobacter* spp. isolates employed in this investigation.

FlaA SVR Allele	Sample Number	Sample Origin
	11695	Parent Breeder Fecal
	11382	Parent Breeder Fecal
	12435	Parent Breeder Fecal
	12250	Parent Breeder Fecal
	5834	Retail Product
	9465	Retail Product
	13601	Retail Product
DAC	13262	Broiler Caeca
	14641	Wild Birds
	13280	Broiler Caeca
	13931	Parent Breeder Fecal
	13926	Parent Rearing Fecal
DAD	11623	Domestic Species
	13769	Human Isolate
	13783	Human Isolate
	12572	Other Poultry
	14118	Human Isolate
	12537	Other Poultry
	7358	Parent Breeder Fecal
	8889	Parent Breeder Fecal
	7571	Parent Breeder Fecal
	13147	Retail Product
	9522	Retail Product

Table 4.2. Genes identified as absent* from *C. jejuni* isolate 14118 (high invasion) based on microarray hybridization analysis with *C. jejuni* 11168 (PMSRU).

Gene	Function
cj1273c	*rpoZ* :DNA-directed RNA polymerase subunit omega, promotes RNA polymerase assembly
cj0860	probable integral membrane protein, permeases of the drug/metabolite transporter (DMT) superfamily
cj0567	hypothetical protein
cj0407	*lgt* :prolipoprotein diacylglyceryl transferase, cell wall/membrane/envelope biogenesis
cj0424	putative acidic periplasmic protein, uncharacterized protein conserved in bacteria
cj1331	*ptmB* :acylneuraminate cytidylyltransferase, flagellin modification
cj0668	putative ATP /GTP-binding protein
cj0753c	*tonB3* :transport protein, links inner and outer membranes
cj0797c	hypothetical protein
cj0436	hypothetical protein
cj0378c	hypothetical protein

*Gack values were ≥-0.05, indicative of absence relative to *C. jejuni* 11168

Table 4.3. Genes identified as absent* from *C. jejuni* isolate 5116 (mid invasion) based on microarray hybridization analysis with *C. jejuni* 11168 (PMSRU).

Name	Function
cj0412	putative ATP /GTP binding protein
cj0010c	*rnhB*: ribonuclease HII, replication, recombination and repair
cj0087	*aspA:* aspartate ammonia-lyase,
cj1249	hypothetical protein
cj1534c	possible bacterioferritin, DNA-binding ferritin-like protein (oxidative damage protectant)
cj1449c	hypothetical protein
cj0266c	putative integral membrane protein
cj0073c	hypothetical protein
cj1210	putative integral membrane protein
cj0065c	*folk:* putative 2-amino-4-hydroxy-6-hydroxymethyldihydropteridine pyrophosphokinase
cj1724c	7-cyano-7-deazaguanine reductase
cj0323	hypothetical protein
cj1587c	multidrug transporter membrane component/ATP-binding component
cj0802	*cysS:* cysteinyl-tRNA synthetase
cj0567	hypothetical protein
cj0262c	putative methyl-accepting chemotaxis signal transduction protein
cj1567c	*nuoM*: NADH dehydrogenase I chain M
cj1642	hypothetical protein

*Gack values were ≥-0.05, indicative of absence relative to *C. jejuni* 11168

Table 4.4. Genes identified as absent* from *C. jejuni* isolate 8557 (mid invasion) based on microarray hybridization analysis with *C. jejuni* 11168 (PMSRU).

Name	Function
cj0056c	hypothetical protein
cj1436c	putative amino transferase
cj0101	*parB*: family protein, predicted transcriptional regulators

*Gack values were ≥-0.05, indicative of absence relative to *C. jejuni* 11168

Table 4.5- Genes identified as absent* from *C. jejuni* isolate 13262 (low invasion) based on microarray hybridization analysis with *C. jejuni* 11168 (PMSRU).

Name	Function
cj0192c	*clpP:* ATP-dependent Clp protease proteolytic subunit
cj1186c	*petA:* putative ubiquinol-cytochrome C reductase iron-sulfur subunit
cj0894c	*ispH* 4-hydroxy-3-methylbut-2-enyl diphosphate reductase, penicillin tolerance protein
cj1152c	putative phosphatase
cj0567	hypothetical protein
cj0810	*nadE:* putative NH(3)-dependent NAD(+) synthetase
cj1060c	small hydrophobic protein
cj0737	putative periplasmic protein, large exoproteins involved in heme utilization or adhesion
cj1038	probable cell division/peptidoglycan biosynthesis protein
cj1435c	hypothetical protein
cj0407	*Lgt:* prolipoprotein diacylglyceryl transferase
cj1271c	*tyrS:* tyrosyl-tRNA synthetase
cj1255	putative isomerase
cj0267c	putative integral membrane protein
cj0224	*argC:* N-acetyl-gamma-glutamyl-phosphate reductase
cj0695	*ftsA:* cell division protein
cj1016c	*livM:* putative branched-chain amino-acid ABC transport system permease protein
cj0999c	putative integral membrane protein
cj1409	*acpS:* 4'-phosphopantetheinyl transferase
cj1567c	*nuoM:* NADH dehydrogenase I chain M
cj0063c	putative ATP-binding protein
cj0641	hypothetical protein
cj1243	*hemE:* uroporphyrinogen decarboxylase
cj0079c	*cdtA:* cytolethal distending toxin
cj0441	*acpP:* acyl carrier protein
cj0789	putative RNA nucleotidyltransferase
cj1379	*selB:* putative selenocysteine-specific elongation factor
cj0036	hypothetical protein
cj0366c	transmembrane efflux protein
cj0303c	*modA:* putative molybdate-binding lipoprotein
cj0825	putative processing peptidase, Type II secretory pathway
cj0188c	hypothetical protein
cj1181c	*Tsf:* elongation factor Ts

*Gack values were ≥-0.05, indicative of absence relative to *C. jejuni* 11168

Table 4.5 cont. Genes identified as absent* from *C. jejuni* isolate 13262 (low invasion) based on microarray hybridization analysis with *C. jejuni* 11168 (PMSRU).

Name	Function
cj0021c	hypothetical protein
cj0649	hypothetical protein
cj1486c	Putative periplasmic protein
cj1699c	*rpmC:* 50S ribosomal protein L29
cj1548c	putative NADP-dependent alcohol dehydrogenase
cj0172c	hypothetical protein
cj1133	*waaC:* putative lipopolysaccharide heptosyltransferase
cj1533c	putative helix-turn-helix containsing protein
cj0717	hypothetical protein
cj1254	hypothetical protein
cj0055c	hypothetical protein
cj1689c	*rplO:* 50S ribosomal protein L15
cj0087	*aspA:* aspartate ammonia-lyase
cj1436c	putative aminotransferase
cj0017c	putative ATP /GTP binding protein
cj0070c	hypothetical protein
cj0805	putative zinc protease
cj0056c	hypothetical protein
cj1200	putative periplasmic protein
cj1069	hypothetical protein
cj0897c	*pheS:* phenylalanyl-tRNA synthetase subunit alpha
cj1449c	hypothetical protein
cj1636c	*rnhA:* ribonuclease H
cj0391c	hypothetical protein
cj0516	*plsC:* putative 1-acyl-SN-glycerol-3-phosphate acyltransferase
cj1491c	putative two-component regulator
cj0487	hypothetical protein
cj0461c	putative integral membrane protein
cj0724	hypothetical protein
cj0428	hypothetical protein
cj0352	putative transmembrane protein
cj0932c	*pckA:* phosphoenolpyruvate carboxykinase
cj1021c	putative periplasmic protein

*Gack values were ≥-0.05, indicative of absence relative to *C. jejuni* 11168

Table 4.6: Genes determined to be commonly distributed throughout *C. jejuni* isolates 14118, 5116, 8557 and 13262 based on microarray hybridization with *C. jejuni* 11168 (PMSRU).

Gene ID	Description
Amino acid transport and metabolism	
cj0764c	*speA:* arginine decarboxylase
cj1018c	branched-chain amino-acid ABC transport system periplasmic binding protein
cj1502c	*putP:* sodium/proline symporter
cj0931c	*argH:* argininosuccinate lyase
cj1378	*selA:* selenocysteine synthase
cj0922c	*pebC:* ABC-type amino-acid transporter ATP-binding protein
cj1580c	putative peptide ABC-transport system ATP-binding protein
cj1286	*upp:* uracil phosphoribosyltransferase
cj0762c	*aspB:* aspartate aminotransferase
cj0481	putative lyase
cj0227	*argD:* acetylornithine aminotransferase
cj1315c	*hisH:* imidazole glycerol phosphate synthase subunit HisH
cj0314	*lysA:* diaminopimelate decarboxylase
cj1202	*metF:* 5,10-methylenetetrahydrofolate reductase
cj0317	histidinol-phosphate aminotransferase
cj0734c	*hisJ:* histidine-binding protein precursor
cj0349	*trpA:* tryptophan synthase subunit alpha
cj0716	putative phospho-2-dehydro-3-deoxyheptonate aldolase
cj1605c	*dapD:* possible 2,3,4,5-tetrahydropyridine-2-carboxylate N-succinyltransferase
cj0574	*ilvI:* acetolactate synthase 3 catalytic subunit
cj0197c	*dapB:* dihydrodipicolinate reductase
cj0817	*glnH:* glutamine-binding periplasmic protein
cj1014c	*livF:* branched-chain amino-acid ABC transport system ATP-binding protein
cj0980	putative peptidase
cj1624c	*sdaA:* L-serine dehydratase
cj1601	*hisA:* phosphoribosylformimino-5-aminoimidazole carboxamide ribotide isomerase

Table 4.6 cont. Genes determined to be commonly distributed throughout *C. jejuni* isolates 14118, 5116, 8557 and 13262 based on microarray hybridization with *C. jejuni* 11168 (PMSRU).

Gene ID	Description
cj0149c	*hom:* homoserine dehydrogenase
cj1598	*hisD:* histidinol dehydrogenase
cj0240c	cysteine desulfurase
cj0940c	*glnP:* putative glutamine transport system permease
cj0632	*ilvC:* ketol-acid reductoisomerase
cj0130	*tyrA:* prephenate dehydrogenase
cj1314c	putative cyclase
cj0921c	bifunctional adhesin/ABC transporter aspartate/glutamate-binding protein
cj0609c	Possible periplasmic protein
cj0665c	*argG:* argininosuccinate synthase
Nucleotide transport and metabolism	
cj1498c	adenylosuccinate synthetase
cj0353c	phosphatase
cj1195c	*pyrC2:* dihydroorotase
cj0953c	*purH:* bifunctional phosphoribosylaminoimidazolecarboxamide formyltransferase/IMP cyclohydrolase
cj0117	pfs 5'-methylthioadenosine/S-adenosylhomocysteine nucleosidase
cj0196c	*ppurF:* amidophosphoribosyltransferase
cj0419	hypothetical protein
Carbohydrate transport and metabolism	
cj0339	putative transmembrane transport protein
cj1588	putative transmembrane transport protein
cj0250c	putative transmembrane transport protein
cj1174	putative efflux protein
cj1588c	putative transmembrane transport protein
cj1619	*kgtP:* alpha-ketoglutarate permease
cj1401c	*tpiA:* triosephosphate isomerase
cj1645	*tkt:* transketolase
cj0128c	suhB like protein
cj0486	putative sugar transporter

Table 4.6 cont. Genes determined to be commonly distributed throughout *C. jejuni* isolates 14118, 5116, 8557 and 13262 based on microarray hybridization with *C. jejuni* 11168 (PMSRU).

Gene ID	Description
cj0392c	*pyk*: pyruvate kinase
cj1597	*hisG*: ATP phosphoribosyltransferase
cj1418c	hypothetical protein
Cell cycle control, cell division, chromosome partitioning	
cj0886c	*ftsK*: putative cell division protein
cj0696	*ftsZ*: cell division protein FtsZ
cj1606c	*Mrp*: putative ATP/GTP-binding protein (mrp protein homolog)
Cell Motility	
cj1729c	*flgE*: flagellar hook protein
cj0043	*FlgE*: flagellar hook protein
cj0528c	*flgB*: flagellar basal body rod protein
cj1338c	*flaB*: flagellin
cj1675	*fliQ*: flagellar biosynthesis protein
cj0318	*fliF* :flagellar MS-ring protein
cj0060c	*fliM*: flagellar motor switch protein
cj0059c	*fliY*: flagellar motor switch protein
cj0882c	*flhA*: flagellar biosynthesis protein A
cj0887c	*flaD*: flagellar hook-associated protein
cj0526c	*fliE*: flagellar hook-basal body protein
cj0283c	*cheW*: chemotaxis protein
cj0064c	*flhF*: flagellar biosynthesis regulator FlhF
cj0697	*flgG2*: putative flagellar basal-body rod protein
cj1190c	putative MCP-domain signal transduction protein
cj0924c	*cheB*: putative MCP protein-glutamate methylesterase
cj1474c	putative type II protein secretion system D protein
cj1471c	putative type II protein secretion system E protein
cj0246c	putative MCP-domain signal transduction protein

Table 4.6 cont. Genes determined to be commonly distributed throughout *C. jejuni* isolates 14118, 5116, 8557 and 13262 based on microarray hybridization with *C. jejuni* 11168 (PMSRU).

Gene ID	Description
cj1343c	putative periplasmic protein
Cell Wall/membrane/envelope biogenesis	
cj0910	putative periplasmic protein
cj0131	putative periplasmic protein
cj0735	putative periplasmic protein
cj1406c	putative periplasmic protein
cj0129c	outer membrane protein
cj1670c	putative periplasmic protein
cj1621	putative periplasmic protein
cj1513c	possible periplasmic protein
cj0365c	putative outer membrane channel protein
cj0367c	putative membrane fusion component of efflux system
cj0162c	putative periplasmic protein
cj1416c	putative sugar nucleotidyltransferase
cj1289	possible periplasmic protein
cj1485c	putative periplasmic protein
cj0413	putative periplasmic protein
cj0770c	putative periplasmic protein
cj1428c	*fcl*: putative fucose synthetase
cj1142	*neuC1*: putative N-acetylglucosamine-6-phosphate 2-epimerase/N-acetylglucosamine-6-phosphatase
cj0511	putative secreted protease
cj1151c	*waaD*: ADP-L-glycero-D-manno-heptose-6-epimerase
cj1131c	UDP-glucose 4-epimerase
cj1317	N-acetylneuraminic acid synthetase
cj0821	glmU UDP-N-acetylglucosamine pyrophosphorylase
cj0645	putative secreted transglycosylase

Table 4.6 cont. Genes determined to be commonly distributed throughout *C. jejuni* isolates 14118, 5116, 8557 and 13262 based on microarray hybridization with *C. jejuni* 11168 (PMSRU).

Gene ID	Description
cj0576	*lpxD*: UDP-3-O-[3-hydroxymyristoyl] glucosamine N-acyltransferase
cj1294	putative aminotransferase (degT family)
cj1128	*wlaD*: putative glycosyltransferase
cj1311	acylneuraminate cytidylyltransferase
cj0611c	putative transmembrane transport protein
cj1055c	putative integral membrane protein
cj0238	putative integral membrane protein
cj0946	putative lipoprotein
cj0361	*lspA*: lipoprotein signal peptidase
cj1030c	*lepA*: GTP-binding protein LepA
cj1086c	hypothetical protein
Coenzyme transport and metabolism	
cj0542	*hemA*: glutamyl-tRNA reductase
cj0857c	*moeA*: putative molybdopterin biosynthesis protein
cj0580c	coproporphyrinogen III oxidase
cj0580c	coproporphyrinogen III oxidase
cj0853c	*hemL*: glutamate-1-semialdehyde aminotransferase
cj0725c	*mogA*: molybdenum cofactor biosynthesis protein
cj1218c	*ribA*: riboflavin synthase subunit alpha
cj1046c	thiamine biosynthesis protein ThiF
cj1239	*pdxA*: 4-hydroxythreonine-4-phosphate dehydrogenase
cj0306c	*bioF*: 8-amino-7-oxononanoate synthase
cj0589	*ribF*: bifunctional riboflavin kinase/FMN adenylyltransferase
cj0308c	*bioD*: putative dethiobiotin synthetase

Table 4.6 cont. Genes determined to be commonly distributed throughout *C. jejuni* isolates 14118, 5116, 8557 and 13262 based on microarray hybridization with *C. jejuni* 11168 (PMSRU).

Gene ID	Description
cj0585	*folP*: putative dihydropteroate synthase
cj0453	*thiC*: thiamine biosynthesis protein ThiC
cj0383c	*ribH*: riboflavin synthase subunit beta
cj1458c	*thiL*: thiamine monophosphate kinase
cj0230c	nicotinate phosphoribosyltransferase
cj1368	hypothetical protein
cj1404	hypothetical protein
cj1047c	hypothetical protein
Defense mechanism	
cj0077c	*cdtC*: cytolethal distending toxin
cj0205	*bacA*: putative undecaprenol kinase
cj0619	putative integral membrane protein
cj0140	hypothetical protein
Energy production and conversion	
cj1382c	*fldA*: flavodoxin FldA
cj1265c	*hydC*: Ni/Fe-hydrogenase B-type cytochrome subunit
cj1066	*rdxA*: nitroreductase
cj0537	*oorB*: 2-oxoglutarate-acceptor oxidoreductase subunit OorB
cj0439	*sdhC*: putative succinate dehydrogenase subunit C
cj0076c	L-lactate permease
cj0333c	*fdxA*: ferredoxin
cj0409	fumarate reductase
cj1586	putative bacterial haemoglobin
cj0074c	putative iron-sulfur protein
cj0991c	putative oxidoreductase ferredoxin-type electron transport protein
cj1488c	*ccoQ*: cb-type cytochrome C oxidase subunit IV
cj0781	*napG*: quinol dehydrogenase periplasmic component
cj1570c	*nuoJ*: NADH dehydrogenase subunit J

Table 4.6 cont. Genes determined to be commonly distributed throughout *C. jejuni* isolates 14118, 5116, 8557 and 13262 based on microarray hybridization with *C. jejuni* 11168 (PMSRU).

Gene ID	Description
cj1578c	*nuoB*: NADH dehydrogenase subunit B
cj0783	*napB*: periplasmic nitrate reductase small subunit
cj0265c	putative cytochrome C-type haem-binding periplasmic protein
cj1267c	Ni/Fe-hydrogenase small chain
cj0104	*atpH*: F0F1 ATP synthase subunit delta
cj1192	putative C4-dicarboxylate transport protein
cj1167	*ldh*: putative L-lactate dehydrogenase
cj0532	malate dehydrogenase
cj0780	*napA*: periplasmic nitrate reductase
cj1399c	putative Ni/Fe-hydrogenase small subunit
cj1153	putative periplasmic cytochrome C
cj0203	putative transmembrane protein
Intercellular trafficking, secretion and vesicular transport	
cj0986c	putative integral membrane protein
cj0530	putative periplasmic protein
cj0851c	putative integral membrane protein
cj0852c	putative integral membrane protein
cj0587	putative integral membrane protein
cj1092c	*secF*: preprotein translocase subunit *SecF*
cj1206c	*ftsY*: putative signal recognition particle protein
cj0110	*exbD*: tolR family transport protein
cj0579c	sec-independent translocase
cj0472	*secE* preprotein translocase subunit *SecE*
cj0578c	sec-independant protein translocase
Lipid transport and metabolism	
cj0375	putative lipoprotein

Table 4.6 cont. Genes determined to be commonly distributed throughout *C. jejuni* isolates 14118, 5116, 8557 and 13262 based on microarray hybridization with *C. jejuni* 11168 (PMSRU).

Gene ID	Description
cj1090c	putative lipoprotein
cj0176c	putative lipoprotein
cj0158c	putative haem-binding lipoprotein
cj0978c	putative lipoprotein
cj1299	putative acyl ccarrier protein
cj1029c	*mapA*: putative lipoprotein
cj1279c	putative fibronectin domain-containing lipoprotein
cj0842	putative lipoprotein
cj1346c	*dxr*: 1-deoxy-D-xylulose 5-phosphate reductoisomerase
cj1665	possible lipoprotein thiredoxin
cj1026c	putative lipoprotein
cj1104	4-diphosphocytidyl-2-C-methyl-D-erythritol kinase
cj1347c	*cdsA*: phosphatidate cytidylyltransferase
cj0329c	*plsX*: fatty acid/phospholipid synthesis protein
cj1037c	*pycA*: acetyl-CoA carboxylase
cj0182	putative transporter
Posttranslational modification, protein turnover, and chaperones	
cj0759	*dnaK*: molecular chaperone DnaK
cj1207c	putative lipoprotein thiredoxin
cj0193c	*tig*: trigger factor
cj1112c	methionine sulfoxide reductase B
cj0623	*hypB*: hydrogenase isoenzyme formation protein
cj0596	*peb4*:cbf2 peptidyl-prolyl cis-trans isomerase
cj1106	possible periplasmic thioredoxin
cj0954c	putative dnaJ-like protein
cj0622	hypF: transcriptional regulatory protein hypF

Table 4.6 cont. Genes determined to be commonly distributed throughout *C. jejuni* isolates 14118, 5116, 8557 and 13262 based on microarray hybridization with *C. jejuni* 11168 (PMSRU).

Gene ID	Description
cj1639	*nifU*: protein homolog
cj0701	putative protease
cj0133	hypothetical
cj0625	*hypD*: hydrogenase isoenzyme formation protein
cj1035c	arginyl-tRNA-protein transferase
cj1034c	possible dnaJ-like protein
cj1289	possible periplasmic protein
cj0950c	putative lipoprotein
Signal transduction mechanisms	
cj1024c	signal-transduction regulatory protein
cj0890c	putative sensory transduction transcriptional regulator
cj0248	hypothetical protein
cj0643	putative two-component response regulator
cj1261	two-component regulator
cj1222c	putative two-component sensor
Replication, recombination and repair	
cj0718	*dnaE*: DNA polymerase III subunit alpha
cj0002	*dnaN*: DNA polymerase III subunit beta
cj0464	*recG*: ATP-dependent DNA helicase
cj1157	DNA polymerase III subunits gamma and tau
cj0003	*gyrB*: DNA gyrase subunit B
cj0001	*dnaA*: chromosomal replication initiation protein
cj0338c	*polA*: DNA polymerase I
cj0198c	recombination factor protein *RarA*
cj0836	*ogt*: methylated-DNA--protein-cysteine methyltransferase
cj0595c	endonuclease III
cj0680c	*uvrB*: excinuclease ABC subunit B

Table 4.6 cont. Genes determined to be commonly distributed throughout *C. jejuni* isolates 14118, 5116, 8557 and 13262 based on microarray hybridization with *C. jejuni* 11168 (PMSRU).

Gene ID	Description
Transcription	
cj1156	*rho:* transcription termination factor Rho
cj0368c	transcriptional regulatory protein
cj1563c	putative transcriptional regulator
cj1230	*hspR*: putative heat shock transcriptional regulator
cj0394c	pantothenate kinase
cj1635c	*rnc*: ribonuclease III
cj0478	*rpoB*: DNA-directed RNA polymerase subunit beta
cj0883c	hypothetical protein
Translation ribosomal structure and biogensis	
cj0207	*infC*: translation initiation factor IF-3
cj0094	*rplU*: 50S ribosomal protein L21
cj0474	*rplK*: 50S ribosomal protein L11
cj1182c	*rpsB*: 30S ribosomal protein S2
cj1592	*rpsM*: 30S ribosomal protein S13
cj1694c	*rpsN* :30S ribosomal protein S14
cj1701c	*rpsC*: 30S ribosomal protein S3
cj0884	*rpsO*: 30S ribosomal protein S15
cj1702c	*rplV*: 50S ribosomal protein L22
cj1696c	*rplX*: 50S ribosomal protein L24
cj0370	*rpsU*: 30S ribosomal protein S21
cj1692c	*rplF*: 50S ribosomal protein L6
cj0476	*rplJ*: 50S ribosomal protein L10
cj1697c	*rplN*: 50S ribosomal protein L14
cj0640c	*aspS*: aspartyl-tRNA synthetase
cj0765c	*hisS*: histidyl-tRNA synthetase
cj0845c	*gltX*: glutamyl-tRNA synthetase
cj0577c	*queA*: S-adenosylmethionine:tRNA ribosyltransferase-isomerase
cj0153c	putative rRNA methylase

Table 4.6 cont. Genes determined to be commonly distributed throughout *C. jejuni* isolates 14118, 5116, 8557 and 13262 based on microarray hybridization with *C. jejuni* 11168 (PMSRU).

Gene ID	Description
cj0636	NOL1\NOP2\sun family protein
cj0166	*miaA*: tRNA delta(2)-isopentenylpyrophosphate transferase
cj0930	translation-associated GTPase
cj0588	*tlyA*: putative haemolysin
cj0879c	putative periplasmic protein
Inorganic ion transport and metabolism	
cj0755	*cfrA*: putative iron uptake protein
cj1354	enterochelin uptake ATP-binding protein
cj0237	carbonic anyhydrase
cj1194	possible phosphate permease
cj0614	*pstC*: putative phosphate transport system permease protein
cj0785	possible *napD* protein homolog
cj1284	*ktrA*: putative K+ uptake protein
cj1283	*ktrB*: putative K+ uptake protein
cj1398	*feoB*: ferrous iron transport protein
cj0612c	*cft*: ferritin
cj1615	putative haemin uptake system permease protein
cj0616	putative phosphate transport ATP-binding protein
cj0263	zinc transporter *ZupT*
cj0020c	cytochrome C551 peroxidase
cj0045c	putative iron-binding protein
cj1617	*chuD*: putative haemin uptake system periplasmic haemin-binding protein
cj1614	haemin uptake system outer membrane receptor
cj0142c	ABC transporter ATP-binding protein
cj0141c	ABC transporter integral membrane protein
cj0169	*sodB*: superoxide dismutase
cj0772c	putative periplasmic protein
cj0613	*pstS*: possible periplasmic phosphate binding protein
cj0770c	putative periplasmic protein
cj1658	putative integral membrane protein

Table 4.6 cont. Genes determined to be commonly distributed throughout *C. jejuni* isolates 14118, 5116, 8557 and 13262 based on microarray hybridization with *C. jejuni* 11168 (PMSRU).

Gene ID	Description
cj0241c	putative iron-binding protein
Secondary metabolite biosynthesis and transport	
cj0261c	hypothetical protein
cj0977	hypothetical protein
cj0590	hypothetical protein
General Function prediction only	
cj0572	*ribA:* bifunctional 3,4-dihydroxy-2-butanone 4-phosphate synthase/GTP cyclohydrolase II protein
cj0431	putative periplasmic ATP /GTP-binding protein
cj1041c	putative periplasmic ATP/GTP-binding protein
cj1159c	small hydrophobic protein
cj1545c	*MdaB:* protein homolog
cj0834c	ankyrin repeat-containing possible periplasmic protein
cj0154c	putative methylase
cj1278c	*trmB:* tRNA (guanine-N(7))-methyltransferase
cj1321	putative transferase
cj0778	major antigenic peptide PEB2
cj0947c	putative hydrolase
cj0985c	hippurate hydrolase
cj0900c	small hydrophobic protein
cj0251c	highly acidic protein
cj0556	hypothetical protein
cj1270c	hypothetical protein
cj0760	hypothetical protein
cj0465c	hypothetical protein
cj1225	hypothetical protein
cj0647	hypothetical protein
cj1307	putative amino acid activating enzyme
cj0935c	putative transmembrane transport protein
cj0183	putative integral membrane protein with haemolysin domain

Table 4.6 cont. Genes determined to be commonly distributed throughout *C. jejuni* isolates 14118, 5116, 8557 and 13262 based on microarray hybridization with *C. jejuni* 11168 (PMSRU).

Gene ID	Description
cj1373	putative integral membrane protein
cj0846	putative integral membrane protein
cj0091	putative lipoprotein
cj0413	putative periplasmic protein
Unknown function	
cj0993c	hypothetical protein
cj0254	hypothetical protein
cj0794	hypothetical protein
cj1475c	hypothetical protein
cj1209	hypothetical protein
cj0138	hypothetical protein
cj0796c	hypothetical protein
cj0569	hypothetical protein
cj1144c	hypothetical protein
cj1162c	hypothetical protein
cj0877c	hypothetical protein
cj0494	hypothetical protein
cj0403	hypothetical protein
cj0286c	hypothetical protein
cj1232	hypothetical protein
cj1484c	hypothetical protein
cj1214c	hypothetical protein
cj1245c	hypothetical protein
cj1176c	hypothetical protein
cj1562	hypothetical protein
cj0522	hypothetical protein
cj0959c	hypothetical protein
cj1012c	hypothetical protein
cj1631c	hypothetical protein
cj0963	hypothetical protein

Table 4.6 cont. Genes determined to be commonly distributed throughout *C. jejuni* isolates 14118, 5116, 8557 and 13262 based on microarray hybridization with *C. jejuni* 11168 (PMSRU).

Gene ID	Description
cj1006c	hypothetical protein
cj0700	hypothetical protein
cj1467	hypothetical protein
cj0189c	hypothetical protein
cj1453c	hypothetical protein
cj0247c	hypothetical protein
cj0815	hypothetical protein
cj0598	hypothetical protein
cj1405	hypothetical protein
cj1575c	hypothetical protein
cj1465	hypothetical protein
cj0800c	hypothetical protein
cj0041	hypothetical protein
cj1236	hypothetical protein
cj0418	hypothetical protein
cj0455c	hypothetical protein
cj1656c	hypothetical protein
cj0583	hypothetical protein
cj1384c	hypothetical protein
cj0849c	hypothetical protein
cj0563	hypothetical protein
cj1089c	hypothetical protein
cj0550	hypothetical protein
cj0302c	hypothetical protein
cj0873c	hypothetical protein
cj1443c	*KpsF*: protein
cj0552	hyprophobic protein

Table 4.6 cont. Genes determined to be commonly distributed throughout *C. jejuni* isolates 14118, 5116, 8557 and 13262 based on microarray hybridization with *C. jejuni* 11168 (PMSRU).

Gene ID	Description
cj0610c	putative periplasmic protein
cj0593c	putative integral membrane protein
cj0204	putative integral membrane protein
cj0553	putative integral membrane protein
cj1166c	putative integral membrane protein
cj0014c	putative integral membrane protein
cj1022c	putative integral membrane protein

Table 4.7. Total number of inserts and clones provided for sequence analysis along with percentage of clones with sequences absent from *C. jejuni* 11168 (PMSRU) based on suppressive subtractive hybridizations.

Isolate	# of inserts analyzed	# of subtracted clones provided for DNA sequence analysis	% of clones with sequences determined unique relative to 11168 (PMSRU)
13262	94	59	63%
8557	141	79	56%
14118	103	68	66%
5116	33	24	72%

Table 4.8. Isolate 14118 (Caco-2 high invasion) unique clones relative to *C. jejuni* 11168 (PMSRU).

Category & Clone #	Length (bp)	% G+C	BLASTN and BLASTX results [a]	Organism	% Similarity % Identity	Accession no.
Cell Envelop & Surface structures						
37	343	32.4	a) LOS gene locus, partial sequence	*C. jejuni* strain LC	266/266 (100%)	gb|DQ535892.1
			b) unknown	*C. jejuni*	68/68 (100%)	gb|ABZ79836.1
45	221	33.5	a) putative outer-membrane protein	*C. jejuni* 81116	130/131 (99%)	gb|CP000814.1
			b) putative outer-membrane protein	*C. jejuni* 81116	43/48 (89%)	ref|YP_001482042.1
48	492	36.4	a) no significant similarity to any nucleic acid			
			b) putative sugar transferase,	*C. jejuni*	48/73 (65%)	emb|CAI38725.1
57	247	35.2	a) class H lipooligosaccharide biosynthesis gene locus, partial sequence	*C. jejuni* strain RM1553	165/165 (100%)	gb|EU404106.1
			b) unknown	*C. jejuni*	54/54 (100%)	gb|ABZ79829.1
92	322	42.8	a) *flaA* and *flaB* genes	*C.jejuni* TGH9011(ATCC43431)	221/241 (91%)	Z29327.1
			b) *FlaB*	*C. jejuni*	79/80 (98%)	gb|ABS89177.1
130	470	38.1	a) putative integral membrane protein	*C. jejuni* 81116	209/210 (99%)	gb|CP000814.1
			b) putative integral membrane protein	*C. jejuni* 81116	46/47 (97%)	ref|YP_001481584.1
132	308	35.7	a) no significant similarity to any nucleic acid			
			b) putative glycosyltransferase,	*C. jejuni*	17/70 (24%)	b|AAR98510.1
150	253	33.5	a) no significant similarity to any nucleic acid			
			b) motility accessory factor	*C. doylei* 269.97	39/51 (76%)	ref|YP_001397577.1
163	297	37.3	a) putative integral membrane protein,	*C. jejuni* 81116	221/221 (100%)	gb|CP000814.1
			b) putative integral membrane protein,	*C. jejuni* 81116	73/74 (98%)	ref|YP_001481894.1
179	297	36.3	a) 324 bp at 5' side: transformation system protein, 738 bp at 3' side: motility accessory factor	*C. doylei* 269.97	185/199 (92%)	gb|CP000768.1
			b) hypothetical protein C8J_1258	*C. jejuni* 81116	62/70 (88%)	ref|YP_001482834.1
191	375	33.0	a) class O lipooligosaccharide biosynthesis gene locus, partial sequence	*C. jejuni* RM3423	294/298 (98%)	gb|EF143352.1
			b) putative dTDP-glucose 4,6-dehydratase	*C. jejuni*	76/79 (96%)	gb|ABN41486.1

[a] a) BLASTN hit and b) BLASTX hit

Table 4.8 cont. Isolate 14118 (Caco-2 high invasion) unique clones relative to *C. jejuni* 11168 (PMSRU).

Category & Clone #	Length (bp)	% G+C	BLASTN and BLASTX results [a]	Organism	% Similarity % Identity	Accession no.
197	527	40.9	a) class J lipooligosaccharide biosynthesis gene locus	*C. jejuni* RM1508	224/236 (94%)	gb|EU404104.1
			b) hypothetical protein C8J_1345	*C. jejuni* 81116	78/85 (91%)	YP_001482920.1
209	249	39.7	a) class S lipooligosaccharide biosynthesis gene locus, partial sequence	*C. jejuni* RM3419	156/158 (98%)	gb|EU404110.1
			b) unknown	*C. jejuni*	40/44 (90%)	gb|ABZ79851.1
229	315	32.6	a) class H lipooligosaccharide biosynthesis gene locus	*C. jejuni* RM1553	237/239 (99%)	gb|EU404106.1
			b) unknown	*C. jejuni*	78/80 (97%)	gb|ABZ79837.1
241	281	33.4	a) LOS biosynthesis cluster	*C. jejuni* 11828	191/191 (100%)	gb|AF343914.1
			b) hypothetical protein C8J_1094	*C. jejuni* 81116	63/63 (100%)	YP_001482670.1
249	274	43.5	a) flagellin A (*flaA*) gene	*C. jejuni* D5477	148/163 (90%)	gb|AF369587.1
			b) flagellin A	*C. jejuni* HB93-13	57/61 (93%)	ZP_01071151.1
271	168	48.8	a) putative periplasmic protein	*C. jejuni* 81-176	89/92 (96%)	gb|CP000538.1
			b) putative periplasmic protein	*C. jejuni* 81-176	29/32 (90%)	YP_001000654.1
290	268	37.6	a) integral membrane protein gene	*C. jejuni*	190/190 (100%)	gb|AF273109.1
			b) integral membrane protein	*C. jejuni*	62/67 (92%)	gb|AAF82114.1
Restriction-modification, recombination & repair						
26	354	33.6	a) type I restriction-modification system, M subunit	*C. jejuni* 81-176	261/264 (98%)	gb|CP000538.1
			b) type I restriction-modification system, M subunit	*C. jejuni* 81-176	87/91 (95%)	YP_001000444.1
91	294	33.3	a) hypothetical protein	*C. jejuni* 81116	202/204 (99%)	gb|CP000814.1
			b) *RloA*	*C. jejuni*	40/42 (95%)	gb|AAN33168.1
135	392	33.2	a) *RloB*	*C. jejuni* 81116	316/317 (99%)	gb|CP000814.1
			b) *RloB*	*C. jejuni* CG8486	105/106 (99%)	ZP_01809391.1

[a] a) BLASTN hit and b) BLASTX hit

Table 4.8 cont. Isolate 14118 (Caco-2 high invasion) unique clones relative to *C. jejuni* 11168 (PMSRU).

Category & Clone #	Length (bp)	% G+C	BLASTN and BLASTX results [a]	Organism	% Similarity % Identity	Accession no.
173	361	34.9	a) putative McrBC restriction endonuclease system, McrB subunit	*C. doylei* 269.97	211/224 (94%)	gb|CP000768.1
			b) McrBC restriction endonuclease system, McrB subunit, putative	*C. jejuni* HB93-13	82/95 (86%)	ZP_01072052.1
215	320	36.3	a) *HsdR* pseudogene, hsdR-1 allele, complete sequence; *RloG* gene, HsdS pseudogene, hsdS-5 allele, and HsdM gene, hsdM-1 allele	*C. jejuni* RM1167	230/233 (98%)	gb|AF486547.1
			b) *HsdM*	*C. jejuni*	77/77 (100%)	gb|AAM00833.1
224	451	35.6	a) hypothetical protein	*C. jejuni* 81116	279/294 (94%)	gb|CP000814.1
			b) *RloA*	*C. jejuni* CG8486	87/99 (87%)	ZP_01809390.1
291	399	33.3	a) *HsdR* gene, hsdR-1 allele, *RloA* and RloB genes, *HsdS* gene, hsdS-1 allele, and *HsdM* gene, hsdM-1 allele	*C. jejuni* RM1861	262/262 (100%)	gb|AF486553.1
			b) putative type I specificity subunit *HsdS*	*C. jejuni* CG8486	65/65 (100%)	ZP_01809392.1
Transport						
136	403	40.2	a) di-/tripeptide transporter	*C. jejuni* 81116	312/313 (99%)	gb|CP000814.1
			b) di-/tripeptide transporter	*C. jejuni* 81116	106/115 (92%)	YP_001482189.1
225	173	41.6	a) di-/tripeptide transporter	*C. jejuni* 81116	118/118 (100%)	gb|CP000814.1
			b) di-/tripeptide transporter	*C. jejuni* 81116	39/39 (100%)	YP_001482189.1
240	119	36.1	a) Na+/H+ antiporter	*C. jejuni* 81116	41/41 (100%)	gb|CP000814.1
			b) no significant similarity to any protein			
281	102	39.2	a) major facilitator superfamily protein	*C. doylei* 269.97	46/46 (100%)	gb|CP000768.1
			b) major facilitator superfamily protein,	*C. doylei* 269.97	15/15 (100%)	YP_001397475.1
Chemotaxis						
145	412	37.1	a) methyl-accepting chemotaxis protein,	*C. jejuni* 81116	334/336 (99%)	gb|CP000814.1
			b) putative MCP-type signal transduction protein	*C. jejuni*	102/104 (98%)	ZP_01809677.1

[a] a) BLASTN hit and b) BLASTX hit

Table 4.8 cont. Isolate 14118 (Caco-2 high invasion) unique clones relative to *C. jejuni* 11168 (PMSRU).

Category & Clone #	Length (bp)	% G+C	BLASTN and BLASTX results [a]	Organism	% Similarity % Identity	Accession no.
159	432	35.4	a) methyl-accepting chemotaxis protein,	*C. jejuni* 81116	355/355 (100%)	gb|CP000814.1
			b) methyl-accepting chemotaxis protein	*C. jejuni* 81116	110/110 (100%)	YP_001482984.1
Other (bacteriophage sequence)						
234	343	33.8	a) prophage Lp2 protein 6	*C. jejuni* 81-176	255/256 (99%)	gb|CP000538.1
			b) prophage Lp2 protein 6	*C. jejuni* 81-176	85/87 (97%)	ZP_02271034.1
Small molecular metabolism						
82	390	36.9	a) hypothetical protein	*C. jejuni* 81116	185/185 (100%)	gb|CP000814.1
			b) cytochrome c biogenesis protein	*C. jejuni* 81-176	61/61 (100%)	YP_999754.1
30	613	33.5	a), hypothetical protein	*C. jejuni* 81116	371/371 (100%)	gb|CP000814.YP_001481975.1
			b) lipoprotein, putative	*C. jejuni* 81116	47/48 (97%)	
148	644	34.2	a) hypothetical protein	*C. jejuni* 81-176	561/569 (98%)	gb|CP000538.1
			b) putative subunit of dimethyl sulfoxide reductase	*C. jejuni*	113/116 (97%)	gb|AAY53800.1
154	228	41.6	a) Ser/Thr protein phosphatase family protein	*C. jejuni* 81116	139/140 (99%)	gb|CP000814.1
			b) Ser/Thr protein phosphatase family protein	*C. jejuni* 81116	45/47 (95%)	YP_001482369.1
172	347	39.5	a) anaerobic dimethyl sulfoxide reductase chain A	*C. jejuni* 81-176	256/259 (98%)	gb|CP000538.1
			b) hypothetical protein C8J_1482	*C. jejuni* 81116	86/86 (100%)	YP_001483057.1
203	351	34.1	a) hydrolase, carbon-nitrogen family	*C. jejuni* RM1221	261/261 (100%)	gb|CP000025.1
			b) hydrolase, carbon-nitrogen family	*C. jejuni* RM1221	72/75 (96%)	YP_179189.1
264	467	32.3	a) oxidoreductase, molybdopterin binding, putative orotidine 5'-phosphate decarboxylase	*C. jejuni* 81-176	380/380 (100%)	gb|CP000538.1
			b) orotidine 5'-phosphate decarboxylase,	*C. jejuni* 81-176	91/92 (98%)	gb|EAQ73091.1
267	486	37.0	a) arylsulfate sulfotransferase	*C. jejuni* 81-176	401/405 (99%)	gb|CP000538.1
			b) arylsulfate sulfotransferase, degenerate	*C. jejuni* 81-176	134/138 (97%)	YP_001000550.1

[a] a) BLASTN hit and b) BLASTX hit

Table 4.8 cont. Isolate 14118 (Caco-2 high invasion) unique clones relative to *C. jejuni* 11168 (PMSRU).

Category & Clone #	Length (bp)	% G+C	BLASTN and BLASTX results [a]	Organism	% Similarity % Identity	Accession no.
272	209	40.6	a) 2-isopropylmalate synthase	*C. jejuni* 81116	113/113 (100%)	gb|CP000814.1
			b) 2-isopropylmalate synthase	*C. jejuni* 81116	37/37 (100%)	YP_001483199.1
279	97	40.0	a) *RlfA*	*C. jejuni* 81116	41/41 (100%)	gb|CP000814.1
			b) no significant similarity to any protein			
Hypothetical & unknown						
25	239	33.9	a) hypothetical protein	*C. jejuni* 81116	161/162 (99%)	gb|CP000814.1
			b) hypothetical protein C8J_0526	*C. jejuni* 81116	52/55 (94%)	YP_001482102.1
28	281	36.2	a) hypothetical protein	*C. jejuni* 81116	189/190 (99%)	gb|CP000814.1
			b) hypothetical protein C8J_0400	*C. jejuni* 81116	60/66 (90%)	YP_001481976.1
40	401	31.7	a) 318 bp at 5' side: ATP synthase F0 sector C subunit, 798 bp at 3' side: hypothetical protein	*C. jejuni* 81116	311/312 (99%)	gb|CP000814.1
			b) hypothetical protein *C. jejuni*_04900	*C. jejuni* 81-176	70/70 (100%)	ZP_02271300.1
47	695	29.7	a) hypothetical protein	*C. jejuni* 81116	277/277 (100%)	gb|CP000814.1
			b) hypothetical protein C8J_0035	*C. jejuni* 81116	92/92 (100%)	YP_001481613.1
54	232	43.1	a), hypothetical protein	*C. jejuni* 81116	155/156 (99%)	gb|CP000814.1
			b) hypothetical protein C8J_1589	*C. jejuni* 81116	50/52 (96%)	YP_001483163.1
67	381	35.6	a) hypothetical protein	*C. jejuni* 81116	291/291 (100%)	gb|CP000814.1
			b) hypothetical protein C8J_0648	*C. jejuni* 81116	86/87 (98%)	YP_001482224.1
78	438	38.1	a) hypothetical protein	*C. jejuni* 81116	314/328 (95%)	gb|CP000814.1
			b) hypothetical protein C8J_0034	*C. jejuni* 81116	97/109 (88%)	YP_001481612.1
122	307	35.1	a) hypothetical protein	*C. jejuni* 81116	231/231 (100%)	b|CP000814.1
			b) hypothetical protein C8J_0036	*C. jejuni* 81116	77/79 (97%)	YP_001481614.1
134	716	34.9	a) hypothetical protein	*C. jejuni* 81116	640/641 (99%)	gb|CP000814.1
			b) hypothetical protein C8J_0988	*C. jejuni* 81116	213/213 (100%)	YP_001482564.1
147	408	45.8	a) hypothetical protein	*C. jejuni* 81116	284/287 (98%)	gb|CP000814.1
			b) hypothetical protein C8J_0878	*C. jejuni* 81116	92/95 (96%)	YP_001482454.1

[a] a) BLASTN hit and b) BLASTX hit

Table 4.8 cont. Isolate 14118 (Caco-2 high invasion) unique clones relative to *C. jejuni* 11168 (PMSRU).

Category & Clone #	Length (bp)	% G+C	BLASTN and BLASTX results [a]	Organism	% Similarity % Identity	Accession no.
153	386	38.8	a) hypothetical protein	*C. jejuni* 81-176	259/304 (85%)	gb|CP000538.1
			b) hypothetical protein CJE1531	*C. jejuni* RM1221	82/99 (82%)	YP_179516.1
155	264	42.4	a) hypothetical protein	*C. jejuni* 81116	171/171 (100%)	gb|CP000814.1
			b) hypothetical protein C8J_0986	*C. jejuni* 81116	56/57 (98%)	YP_001482562.1
170	268	41.0	a) hypothetical protein	*C. jejuni* 81116	172/172 (100%)	gb|CP000814.1
			b) hypothetical protein C8J_0988	*C. jejuni* 81116	60/64 (93%)	YP_001482564.1
187	329	41.3	a) hypothetical protein	*C. jejuni* 81116	216/253 (85%)	gb|CP000814.1
			b) hypothetical protein Cjejjejuni_07040	*C. jejuni* 81-176	76/84 (90%)	ZP_02271659.1
188	320	33.1	a) hypothetical protein	*C. jejuni* 81116	230/234 (98%)	gb|CP000814.1
			b) hypothetical protein C8J_1619	*C. jejuni* 81116	62/62 (100%)	YP_001483193.1
200	362	38.7	a) hypothetical protein	*C. jejuni* 81116	283/285 (99%)	Gb|CP000814.1
			b) hypothetical protein C8J_0142	*C. jejuni* 81116	79/81 (97%)	YP_001481718.1|
208	507	39.2	a) hypothetical protein	*C. jejuni* 81116	416/417 (99%)	gb|CP000814.1
			b) hypothetical protein C8J_0140	*C. jejuni* 81116	138/139 (99%)	YP_001481716.1
231	435	35.1	a) hypothetical protein	*C. jejuni* 81-176	296/300 (98%)	gb|CP000538.1
			b) hypothetical protein cju10	*C. jejuni* 81-176	81/83 (97%)	gb|ABF83701.1
244	399	35.8	a) conserved hypothetical protein, & DNA gyrase, A subunit	*C. jejuni* 81-176	194/195 (99%), 125/126 (99%)	gb|CP000538.1
			b) conserved hypothetical protein	*C. coli* RM2228	55/55 (100%)	ZP_00370899.1
253	268	38.8	a) hypothetical protein,	*C. jejuni* 81116	190/190 (100%)	gb|CP000814.1
			b) hypothetical protein C8J_0400	*C. jejuni* 81116	62/67 (92%)	YP_001481976.1
277	340	32.9	a) hypothetical protein	*C. jejuni* 81116	249/250 (99%)	gb|CP000814.1
			b) hypothetical protein C8J_0065	*C. jejuni* 81116	74/74 (100%)	YP_001481642.1
278	340	32.9	a) hypothetical protein	C. *jejuni* 81116	249/250 (99%)	gb|CP000814.1
			b) hypothetical protein C8J_0065	*C. jejuni* 81116	74/74 (100%)	YP_001481642.1
286	285	33.5	a) hypothetical protein	*C. jejuni* 81116	196/196 (100%)	Gb|CP000814.1
			b) hypothetical protein CJE0273	*C. jejuni* RM1221	40/48 (83%)	YP_178296.1
287	343	40.6	a) no significant similarity to any nucleic acid			
			b) CDP-abequose synthase	*Blastopirellula marina* DSM 3645	41/94 (43%)	ZP_01090940.1

[a] a) BLASTN hit and b) BLASTX hit

Table 4.9. Unique clones, recovered from suppressive subtractive hybridizations of *C. jejuni* isolate 14118 (Caco-2 high invasion) determined to possess significant similarity to *Campylobacter* spp. other than *C. jejuni* 11168 (PMSRU).

Isolate	Percentage of Clones (n=68)
C. jejuni 81116	52.3%
C. jejuni LC	2.0%
C. jejuni RM1553	3.0%
C. jejuni 43431	2.0%
C. jejuni RM3423	2.0%
C. jejuni RM1508	2.0%
C. jejuni RM3419	2.0%
C. jejuni 11828	2.0%
C. jejuni D5477	2.0%
C. jejuni 81-176	11.0%
C. jejuni RM1167	2.0%
C. jejuni RM1861	2.0%
C. jejuni RM1221	2.0%
C. jejuni	5.0%

Table 4.10. Isolate 5116 (Caco-2 mid invasion) unique clones relative to *C. jejuni* 11168 (PMSRU).

Category & Clone #	Length (bp)	% G+C	BLASTN and BLASTX results [a]	Organism	% Similarity % Identity	Accession no.
Cell Envelop & surface structures						
4	524	29.7	a) lipooligosaccharide biosynthesis gene locus	*C. jejuni* RM3423	446/448 (99%)	gb|EF143352.1
			b) putative glycosyltransferase	*C. jejuni*	108/114 (94%)	gb|ABN41491.
37	261	42.9	a) hypothetical protein	*C. jejuni* 81116	183/183 (100%)	gb|CP000814.1
			b) chimeric flagellin A/B	*C. jejuni*	63/71 (88%)	gb|AAF25214.1|AF202 168_1
59	573	25.0	a) no significant similarities to any nucleic acid	*C. coli* RM2228	66/158 (41%)	
			b) capsular polysaccharide synthesis			ZP_00368108.1
65	395	31.9	a) no significant similarities to any nucleic acid	*C. coli* RM2228	85/106 (80%)	
			b) capsular polysaccharide synthesis			ZP_00368108.1
84	275	38.5	a) cell division protein FtsK, putative	*C. jejuni* 81-176	195/198 (98%)	Gb|CP000538.1
			b) cell division protein FtsK, putative,	*C. jejuni* 81-176	50/50 (100%)	ZP_02271252.1
88	511	34.4	a) no significant similarities to any nucleic acid			
			b) putative periplasmic protein,	*C. jejuni* HB93-13	145/146 (99%)	ZP_01071241.1
90	338	38.5	a) capsular polysaccharide biosynthesis protein	*C. doylei* 269.97	240/261 (91%)	gb|CP000768.1
			b) capsular polysaccharide biosynthesis protein	*C. jejuni* HB93-13	84/97 (86%)	ZP_01071340.1
Restriction – modification, recombination and repair						
55	498	30.5	a) type I restriction modification	*C. jejuni* 81-176	421/422 (99%)	gb|CP000538.1
			b) type I restriction modification DNA specificity domain protein	*C. jejuni* 81-176	140/155 (90%)	YP_001000445.1

[a] a) BLASTN hit and b) BLASTX hit

Table 4.10.cont: Isolate 5116 (Caco-2 mid invasion) unique clones relative to *C. jejuni* 11168 (PMSRU).

Category & Clone #	Length (bp)	% G+C	BLASTN and BLASTX results [a]	Organism	% Similarity % Identity	Accession no.
16	253	39.1	a) 1062 bp at 5' side: MATE efflux family protein 176 bp at 3' side: phosphate ABC transporter, ATP-binding protein	*C. doylei* 269.97	165/177 (93%)	gb|CP000768.1
			b) conserved hypothetical protein,	*C. jejuni* 260.94	57/59 (96%)	ZP_01069942.1
34	257	43.1	a) baseplate assembly protein V, putative	*C. jejuni* RM1221	179/180 (99%)	gb|CP000025.1
			b) baseplate assembly protein V, putative	*C. jejuni* 260.94	60/64 (93%)	ZP_01070038.1
77	253	39.5	a) 1062 bp at 5' side: MATE efflux family protein176 bp at 3' side: phosphate ABC transporter, ATP-binding protein	*C. doylei* 269.97	165/177 (93%)	gb|CP000768.1
			b) conserved hypothetical protein	*C. jejuni* 260.94	57/59 (96%)	ZP_01069942.1
Small Molecule Metabolism						
29	660	41.5	a) rRNA-23S ribosomal RNA	*C. jejuni* RM1221	582/591 (98%)	gb|CP000025.1
			b) conserved hypothetical protein,	*C. jejuni* CF93-6	35/35 (100%)	ZP_01067405.1
64	415	32.5	a), phosphoribosylglycinamide formyltransferase	*C. jejuni* 81-176	338/339 (99%)	gb|CP000538.1
			b) phosphoribosylglycinamide formyltransferase	*C. jejuni* 81-176	112/116 (96%)	YP_999906.1

[a] a) BLASTN hit and b) BLASTX hit

Table 4.10.cont. Isolate 5116 (Caco-2 mid invasion) unique clones relative to *C. jejuni* 11168 (PMSRU).

Category & Clone #	Length (bp)	% G+C	BLASTN and BLASTX results [a]	Organism	% Similarity % Identity	Accession no.
Other (bacteriophage sequence)						
12	463	42.3	a) *Campylobacter* phage CGC-2007 isolate *Cj00-2544 cje0217* gene, partial cds; *cje0218*, *cje0219*, *cje0220*, *cje0221*, *cje0222*, *cje0223*, *cje0224*, *cje0225*, *cje0226*, *cje0227*, *cje0228*, *cje0229*, *cje0230*, and *cje0231* genes, complete cds; and *cje0232* gene, partial cds	*Campylobacter*	373/384 (97%)	gb|EF694687.1
			b) *cje0227*, *Campylobacter* phage CGC-2007]	*Campylobacter*	126/128 (98%)	gb|ABU53861.1
Hypothetical and unknown proteins						
3	570	27.5	a) no significant similarities to any nucleic acid			
			b) conserved hypothetical protein		81/164 (49%)	ZP_01067542.1
10	373	30.7	a) hypothetical protein,	*C. jejuni* 81116	290/290 (100%)	gb|CP000814.1
			b) hypothetical protein C8J_1252	*C. jejuni* 81116	96/97 (98%)	YP_001482828.1

[a] a) BLASTN hit and b) BLASTX hit

Table 4.11. Unique clones, recovered from suppressive subtractive hybridizations, of *C. jejuni* isolate 5116 (Caco-2 mid invasion) determined to possess significant similarity to *Campylobacter* spp. other than *C. jejuni* 11168 (PMSRU).

Isolate	Percentage of Clones (%) (n=16)
C. jejuni RM3423	6.0%
C. jejuni 81116	13.0%
C. jejuni RM2228	13.0%
C. jejuni 81-176	19.0%
C. jejuni HP93-13	6.0%
C. jejuni RM1221	13.0%

Table 4.12. Isolate 8557 (Caco-2 mid invasion) unique clones relative to *C. jejuni* 11168 (PMSRU).

Category & Clone #	Length (bp)	% G+C	BLASTN and BLASTX results [a]	Organism	% Similarity % Identity	Accession no.
Cell Envelop & Surface structures						
31	268	44	a) tail fiber protein H, putative	*C. jejuni* RM 1221	188/191 (98%)	CP000025.1
			b) tail fiber protein H, putative	*C. jejuni* 260.94	63/64 (98%)	YP_178254.1
49	336	34.2	a) No Significant Similarities to any nucleic acid			
			b) putative periplasmic protein	*C. jejuni* HB93-13	85/86 (98%)	ZP_01071241.1
52	415	38.0	a) putative peptide chain release factor 2 (*prfB*) gene, partial cds; hypothetical protein, *CysD* (*cysD*), *CysN* (*cysN*), putative sodium/sulfate symporter, putative adenylylsulfate kinase (*cysC*), hypothetical protein, and putative glycosyltransferase genes; and *cj1457c* gene, partial cds	*C. jejuni* ATCC 43432	329/339 (97%)	AY791516.1
			b) *CysN*	*C. jejuni*	102/105 (97%)	AAX33831.1
63	556	30.5	a) LOS biosynthesis cluster	*C. jejuni* 11828	476/480 (99%)	AF343914.1
			b) unknown,	*C. jejuni*	159/163 (97%)	AAK12964.1
69	244	28.6	a) capsular polysaccharide biosynthesis protein,	*C. doylei* 269.97	292 bits (158%)	CP000768.1
			b) capsular polysaccharide biosynthesis protein	*C. jejuni* HB93-13	58/63 (92%)	ZP_01071278.1
86	566	31.9	a) baseplate assembly protein W	*C. jejuni* RM1221	483/490 (98%)	CP000025.1
			b) putative baseplate assembly protein W	*C. jejuni* RM1221	91/93 (97%)	CAB94938.1
134	396	29.0	a) motility accessory factor	*C. jejuni* 81-176	319/319 (100%)	gb|CP000538.1
			b) motility accessory factor	*C. jejuni* 81-176	90/91 (98%)	YP_001000998.1
135	266	36.5	a) putative peptide chain release factor 2 (*prfB*) gene, partial cds; hypothetical protein, *CysD*, *CysN*, putative sodium/sulfate symporter, putative adenylylsulfate kinase,*cysC*, hypothetical protein, and putative glycosyltransferase genes; and *Cj1457c* gene, partial cds	*C. jejuni* ATCC 43432	187/189 (98%)	gb|AY791516.1
			b) putative sodium/sulfate symporter,	*C. jejuni*	63/66 (95%)	gb|AAX33832.1

[a] a) BLASTN hit and b) BLASTX hit

Table 4.12 cont. Isolate 8557 (Caco-2 mid invasion) unique clones relative to *C. jejuni* 11168 (PMSRU).

Category & Clone #	Length (bp)	% G+C	BLASTN and BLASTX results [a]	Organism	% Similarity % Identity	Accession no.
194	250	42.4	a) class O lipooligosaccharide biosynthesis gene locus	*C. jejuni* RM3423	174/174 (100%)	gb|EF143352.1
			b) putative aminotransferase,	*C. jejuni*	60/70 (85%)	gb|ABN41492.1
200	111	41.4	a) flagellar hook protein	*C. doylei* 269.97	52/53 (98%)	gb|CP000768.1
			b) flagellar hook subunit protein	*C. jejuni* CG8486	18/18 (100%)	ZP_01810497.1
204	381	35.7	a) capsular polysaccharide biosynthesis protein	*C. doylei* 269.97	282/303 (93%)	gb|CP000768.1
			b) capsular polysaccharide biosynthesis protein	*C. jejuni* HB93-13	95/102 (93%)	ZP_01071340.1
213	238	39.5	a) class O lipooligosaccharide biosynthesis gene locus	*C. jejuni* RM3423	160/160 (100%)	gb|CP000814.1
			b) putative glucose-1-phosphate thymidyltransferase,	*C. jejuni*	53/53 (100%)	gb|ABN41485.1
Restriction-modification, recombination & repair						
34	241	34	a) no significant similarities to any nucleic acid			
			b) adenine-specific methyltransferase	*Fusobacterium nucleatum*	34/52 (65%)	NP_602723.1
74	269	39.7	a) prophage MuSo1, F protein, putative	*C. jejuni* RM1221	192/192 (100%)	CP000025.1
			b) prophage MuSo1, F protein, putative	*C. jejuni* RM1221	30/30 (100%)	YP_178274.1
191	493	34.9	a) type I restriction-modification system, M subunit	*C. jejuni* 81116	408/411 (99%)	gb|CP000814.1
			b) type I restriction-modification system, M subunit	*C. jejuni* 81116	137/137 (100%)	YP_001483027.1
220	291	34.0	a) HsdR pseudogene, hsdR-1 allele, complete sequence; RloG gene, complete cds; HsdS pseudogene, hsdS-5 allele, and HsdM gene, hsdM-1 allele	*C. jejuni* RM1167	130/135 (96%)	gb|AF486547.1
			b) no significant similarities to any protein		236/244 (96%)	gb|CP000814.1

[a] a) BLASTN hit and b) BLASTX hit

Table 4.12 cont. Isolate 8557 (Caco-2 mid invasion) unique clones relative to *C. jejuni* 11168 (PMSRU).

Category & Clone #	Length (bp)	% G+C	BLASTN and BLASTX results [a]	Organism	% Similarity % Identity	Accession no.
236	791	34.4	a) *HsdR* gene, hsdR-1 allele, complete cds; *RloD* gene, complete cds; *HsdS* gene, *hsdS*-3 allele, complete cds; *MloA* gene, complete cds; and *HsdS* gene, *hsdM*-1 allele, complete cds	*C. jejuni* RM2240	708/715 (99%)	gb|AF486556.1
			b) *HsdM*	*C. jejuni*	236/238 (99%)	gb|AAM00874.1
285	411	31.3	a) type I restriction-modification system, M subunit	*C. jejuni* 81-176	306/338 (90%)	gb|CP000538.1
			b) putative restriction enzyme subunit S	*C. jejuni* 260.94	110/111 (99%)	ZP_01070278.1
Transport						
46	343	39	a) Na/Pi-cotransporter, putative	*C. jejuni* 81-176	252/255 (98%)	CP000538.1
			b) Na/Pi-cotransporter, putative	*C. jejuni* HB93-13	51/53 (96%)	ZP_01071640.1
81	725	30.0	a) Na/Pi-cotransporter, putative	*C. jejuni* 81-176	120/121 (99%)	CP000538.1
			b) predicted ATP-dependent endonuclease of the OLD family	*C. jejuni* 260.94	153/155 (98%)	ZP_01070305.1
126	402	30.8	a) permease, putative	*C. jejuni* RM1221	324/327 (99%)	gb|CP000025.1
			b) hypothetical protein Cj8486_1595c	*C. jejuni* CG8486	24/25 (96%)	ZP_01809396.1
214	726	37.2	a) Na/Pi-cotransporter, putative	*C. jejuni* 81-176	632/634 (99%)	gb|CP000538.1
			b) putative penicillin-binding protein	*C. jejuni* 81116	131/138 (94%)	YP_001482062.1
219	382	35.6	a) putative peptide chain release factor 2 (*prfB*) gene, partial cds; hypothetical protein, *CysD*, *CysN*, putative sodium/sulfate symporter, putative adenylylsulfate kinase (*cysC*), hypothetical protein, and putative glycosyltransferase genes, complete cds; and *Cj1457c* gene, partial cds	*C. jejuni* ATCC 43432	284/284 (100%)	gb|AY791516.1
			b) putative sodium/sulfate symporter	*C. jejuni*	94/94 (100%)	gb|AAX33832.1

[a] a) BLASTN hit and b) BLASTX hit

Table 4.12 cont. Isolate 8557 (Caco-2 mid invasion) unique clones relative to *C. jejuni* 11168 (PMSRU).

Category & Clone #	Length (bp)	% G+C	BLASTN and BLASTX results [a]	Organism	% Similarity % Identity	Accession no.
234	356	31.1	a) transporter, putative	*C. jejuni* RM1221	271/279 (97%)	gb|CP000025.1
			b) conserved hypothetical protein	*C. jejuni* 81-176	25/27 (92%)	YP_001001196.1
Small Molecule Metabolism						
41	239	34.3	a) oxidoreductase, putative	*C. jejuni* 81-176	144/150 (96%)	CP000025.1
			b) oxidoreductase, putative	*C. coli* RM2228	49/51 (96%)	YP_179670.1
98	307	27.4	a) lysyl-tRNA synthetase	*C. jejuni* 81116	207/207 (100%)	gb|CP000814.1
			b) lysyl-tRNA synthetase	*C. jejuni* 81116	69/75 (92%)	gb|ABV51975.1
102	445	48.4	a) dipeptidyl-peptidase	*C. jejuni* 81-176	359/362 (99%)	CP000538.1
			b) X-Pro dipeptidyl-peptidase family protein	*C. jejuni* HB93-13	120/121 (99%)	ZP_01071387.1
166	347	40.6	a) dipeptidyl-peptidase,	*C. jejuni* 81-176	257/258 (99%)	gb|CP000538.1
			b) X-Pro dipeptidyl-peptidase family protein	*C. jejuni* HB93-13	79/81 (97%)	ZP_01071387.1
168	233	41.6	a) arylsulfate sulfotransferase	*C. jejuni* 81116	143/144 (99%)	b|CP000814.1
			b) arylsulfate sulfotransferase	*C. jejuni* 81116	47/50 (94%)	YP_001482389.1
181	520	32.8	a) TPR domain protein	*C. jejuni* 81-176	297/301 (98%)	gb|CP000538.1
			b) putative transmembrane protein	*C. jejuni* 81116	76/89 (85%)	YP_001481941.1
183	457	32.0	a) hypothetical protein	*C. jejuni* 81116	353/363 (97%)	b|CP000814.1
			b) lectin C-type domain protein	*C. doylei* 269.97	32/34 (94%)	YP_001398053.1
185	401	38.9	a) histidyl-tRNA synthetase	*C. jejuni* 81-176	300/302 (99%)	b|CP000538.1
			b) histidyl-tRNA synthetase	*C. jejuni* 81116	96/105 (91%)	YP_001482292.1
196	467	40.0	a) molybdopterin-guanine dinucleotide biosynthesis protein MobB	*C. jejuni* 81-176	199/199 (100%)	gb|CP000538.1
			b) molybdopterin-guanine dinucleotide biosynthesis protein MobB	*C. jejuni* 260.94	62/63 (98%)	ZP_01070343.1
208	900	30.3	a) putative aminotransferase (DegT family)	*C. jejuni* 81116	237/246 (96%)	gb|CP000814.1
			b) probable aminotransferase (degT family)	*C. coli* RM2228	44/44 (100%)	ZP_00367343.1
222	425	34.1	a) CrcB heat shock protein Htp	*C. jejuni* 81116	236/244 (96%)	gb|CP000814.1
			b) CRCB protein like protein,	*C. jejuni* CG8486	42/42 (100%)	ZP_01809576.1

[a] a) BLASTN hit and b) BLASTX hit

Table 4.12 cont. Isolate 8557 (Caco-2 mid invasion) unique clones relative to *C. jejuni* 11168 (PMSRU).

Category & Clone #	Length (bp)	% G+C	BLASTN and BLASTX results [a]	Organism	% Similarity % Identity	Accession no.
229	387	36.2	a) putative pyrazinamidase/nicotinamidase	*C. doylei* 269.97	310/310 (100%)	b|CP000768.1
			b) hypothetical protein CJJ81176_0155	*C. jejuni* 81-176	96/96 (100%)	YP_999844.1
231	844	33.4	a) no significant similarities to any nucleic acid			
			b) ATP/GTP-binding protein	*C. jejuni* 260.94	208/216 (96%)	ZP_01070279.1
260	288	34.0	a) no significant similarities to any nucleic acid		7/37 (100%)	gb|AY725194.1
			b) CAAX amino terminal protease family protein	*C. lari* Rm2100	35/40 (87%)	ZP_00369366.1
261	320	43.1	a) MmgE/PrpD family protein	*C. jejuni* 81-176	243/243 (100%)	gb|CP000538.1
			b) MmgE/PrpD family protein	*C. jejuni* 81-176	80/81 (98%)	YP_001482885.1
266	299	37.1	a) host-nuclease inhibitor protein, putative	*C. jejuni* RM1221	182/207 (87%)	gb|CP000025.1
			b) host-nuclease inhibitor protein, putative	*C. jejuni* 260.94	69/70 (98%)	ZP_01069888.1
Other (bacteriophage sequence)						
2	263	37.6	a) bacteriophage DNA transposition protein A, putative	*C. jejuni* RM1221	183/184 (99%)	CP000025.1
			b) bacteriophage DNA transposition protein A, putative	*C. jejuni* CF93-6	61/62 (98%)	ZP_01068156.1
56	228	33.9	a) phage tail protein, putative	*C. jejuni* RM1221	138/139 (99%)	CP000025.1
			b) phage tail protein	*C. jejuni* RM1221	41/42 (97%)	YP_178275.1
123	507	36.8	a) *Campylobacter* phage CGC-2007 isolate *Cj00-3477 cje0217*-like gene, complete sequence; *cje0218*, *cje0219*, *cje0220*, *cje0221*, *cje0222*, *cje0223*, *cje0224*, *cje0225*, *cje0226*, *cje0227*, *cje0228*, *cje0229*, *cje0230*, and *cje0231* genes, complete cds; and *cje0232* gene, partial cds	*Campylobacter*	429/429 (100%)	gb|EF694689.1
			b) *cje0231*, *Campylobacter* phage CGC-2007	*Campylobacter*	142/143 (99%)	gb|ABU53798.1
139	488	37.5	a) downstream insertion site of CMLP1-like temperate bacteriophage	*C. jejuni*	408/410 (98%)	B|EF092316.1
			b) bacteriophage DNA transposition protein A	*C. jejuni* 260.94	133/137 (97%)	ZP_01069915.1

[a] a) BLASTN hit and b) BLASTX hit

Table 4.12 cont. Isolate 8557 (Caco-2 mid invasion) unique clones relative to *C. jejuni* 11168 (PMSRU).

Category & Clone #	Length (bp)	% G+C	BLASTN and BLASTX results [a]	Organism	% Similarity % Identity	Accession no.
162	320	41.8	a) *Campylobacter* phage CGC-2007 isolate *Cj00-3477 cje0217*-like gene, complete sequence; *cje0218*, *cje0219*, *cje0220*, *cje0221*, *cje0222*, *cje0223*, *cje0224*, *cje0225*, *cje0226*, *cje0227*, *cje0228*, *cje0229*, *cje0230*, and *cje0231* genes, complete cds; and *cje0232* gene, partial cds	*Campylobacter*	237/238 (99%)	gb|EF694689.1
			b) *cje0222*, *Campylobacter* phage CGC-2007	*Campylobacter*	42/43 (97%)	gb|ABU53725.1
177	306	42.4	a) *Campylobacter* phage CGC-2007 isolate *Cj00-3477 cje0217*-like gene, complete sequence; *cje0218*, *cje0219*, *cje0220*, *cje0221*, *cje0222*, *cje0223*, *cje0224*, *cje0225*, *cje0226*, *cje0227*, *cje0228*, *cje0229*, *cje0230*, and *cje0231* genes, complete cds; and *cje0232* gene, partial cds	*Campylobacter*	215/215 (100%)	gb|EF694689.1
			b) *cje0231*, *Campylobacter* phage CGC-2007	*Campylobacter*	73/76 (96%)	gb|ABU53798.1
189	383	37.0	a) *Campylobacter* phage CGC-2007 isolate *Cj00-3477 cje0217*-like gene, complete sequence; *cje0218*, *cje0219*, *cje0220*, *cje0221*, *cje0222*, *cje0223*, *cje0224*, *cje0225*, *cje0226*, *cje0227*, *cje0228*, *cje0229*, *cje0230*, and *cje0231* genes, complete cds; and *cje0232* gene, partial cds	*Campylobacter*	305/307 (99%)	gb|EF694689.1
			b) *cje0231*, *Campylobacter* phage CGC-2007	*Campylobacter*	70/70 (100%)	gb|ABU53865.1
192	250	42.4	a) phage uncharacterized protein	*C. jejuni* RM1221	179/181 (98%)	gb|CP000025.1
			b) phage uncharacterized protein	*C. jejuni* RM1221	25/25 (100%)	YP_178272.1
195	440	32.5	a) *Campylobacter* phage CGC-2007 isolate CjNC13256 *cje0217* gene, partial cds; *cje0218*, *cje0219*, *cje0220*, *cje0221*, *cje0222*, *cje0223*, *cje0224*, *cje0225*, *cje0226*, *cje0227*, *cje0228*, *cje0229*, *cje0230*, and *cje0231* genes, complete cds; and *cje0232* gene, partial cds	*Campylobacter*	355/356 (99%)	gb|EF694693.1
			b) *cje0221*, *Campylobacter* phage CGC-2007	*Campylobacter*	99/100 (99%)	gb|ABU53788.1
202	311	40.3	a) Mu-like prophage I protein	*C. jejuni* RM1221	235/235 (100%)	gb|CP000025.1
			b) Mu-like prophage I protein, putative	*C. jejuni* 260.94	77/77 (100%)	ZP_01069769.1

Table 4.12 cont. Isolate 8557 (Caco-2 mid invasion) unique clones relative to *C. jejuni* 11168 (PMSRU).

Category & Clone #	Length (bp)	% G+C	BLASTN and BLASTX results [a]	Organism	% Similarity % Identity	Accession no.
215	461	41.6	a) *Campylobacter* phage CGC-2007 isolate *Cj00-0949 cje0215* gene, partial cds; *cje0216*, *cje0217*, *cje0218*, *cje0219*, *cje0220*, *cje0221*, *cje0222*, *cje0223*, *cje0224*, *cje0225*, *cje0226*, *cje0227*, *cje0228*, *cje0229*, *cje0230*, and *cje0231* genes, complete cds; and *cje0232* gene, partial cds	*Campylobacter*	375/384 (97%)	gb|EF694684.1
			b) *cje0227*, *Campylobacter* phage CGC-2007	*Campylobacter*	126/128 (98%)	gb|ABU53861.1
250	132	47.7	a) *Campylobacter* phage CGC-2007 isolate *Cj00-2818 cje0215* gene	*Campylobacter*	75/76 (98%)	gb|EF694688.1
			b) *cje0227*, *Campylobacter* phage CGC-2007	*Campylobacter*	25/25 (100%)	gb|ABU53861.1
Hypothetical & unknown proteins						
1	68	34.5	a) no significant similarities to any nucleic acid			
			b) no significant similarities to any protein			
50	402	37.0	a) hypothetical protein	*C. jejuni* 81116	308/325 (94%)	CP000814.1
			b) hypothetical protein CJJ26094_1718	*C. jejuni* 260.94	100/104 (96%)	ZP_01070483.1
82	78	48.7	a) no significant similarities to any nucleic acid			
			b) no significant similarities to any protein			
89	644	36.2	a) hypothetical protein		480/493 (97%)	CP000025.1
			b) conserved hypothetical protein		98/100 (98%)	ZP_01069745.1
114	746	38.0	a) conserved hypothetical protein,	*C. jejuni* 81-176	504/510 (98%)	ZP_01071387.1
			b) hypothetical protein CJJ81176_1327	*C. jejuni* 81-176	169/171 (98%)	gb|CP000538.1
122	214	36.0	a) hypothetical protein	*C. jejuni* 81116	166/175 (94%)	b|CP000814.1
			b) hypothetical protein CJJ26094_1718	*C. jejuni* 260.94	55/58 (94%)	ZP_01070483.
128	505	34.5	a) hypothetical protein	*C. jejuni* RM1221	425/427 (99%)	Gb|CP000025.1
			b) conserved hypothetical protein	*C. jejuni* 260.94	141/142 (99%)	ZP_01069964.1
144	395	38.7	a) conserved hypothetical protein,	*C. jejuni* 81-176	306/306 (100%	gb|CP000538.1
			b) hypothetical protein C8J_0876	*C. jejuni* 81116	101/102 (99%)	YP_001482452.1

[a] a) BLASTN hit and b) BLASTX hit

Table 4.12 cont. Isolate 8557 (Caco-2 mid invasion) unique clones relative to *C. jejuni* 11168 (PMSRU).

Category & Clone #	Length (bp)	% G+C	BLASTN and BLASTX results [a]	Organism	% Similarity % Identity	Accession no.
147	188	47	a) conserved hypothetical protein,	*C. jejuni* 81-176	111/111 (100%)	gb|CP000538.1
			b) hypothetical protein C8J_0878	*C. jejuni* 81116	36/37 (97%)	YP_001482454.1
161	379	39	a) conserved hypothetical protein,	*C. jejuni* 81-176	221/222 (99%)	gb|CP000538.1
			b) hypothetical protein CJJ81176_0761	*C. jejuni* 81-176	74/79 (93%)	YP_001000434.1
172	105	49.5	a) no significant similarities to any nucleic acid			
			b) no significant similarities to any protein			
176	302	37.4	a) hypothetical protein	*C. jejuni* 81116	216/220 (98%)	gb|CP000814.1
			b) hypothetical protein C8J_1093	*C. jejuni* 81116	69/72 (95%)	YP_001482669.1
201	362	34.5	a) hypothetical protein	*C. jejuni* RM1221	271/273 (99%)	Gb|CP000025.1
			b) hypothetical protein CJJ26094_0512	*C. jejuni* 260.94	91/91 (100%)	ZP_01070032.1
203	265	40.3	a) conserved hypothetical protein	*C. jejuni* 81-176	187/188 (99%)	gb|CP000538.1
			b) hypothetical protein C8J_0877	*C. jejuni* 81116	59/62 (95%)	YP_001482453.1
218	215	41.7	a) domain of unknown function (DUF955) superfamily	*C. jejuni* 81116	137/139 (98%)	Gb|CP000814.1
			b) domain of unknown function (DUF955) superfamily	*C. jejuni* 81116	47/48 (97%)	YP_001482223.1
226	567	39.8	a) conserved hypothetical protein	*C. jejuni* RM1221	483/485 (99%)	gb|CP000025.1
			b) hypothetical protein CJE0246	*C. jejuni* RM1221	159/163 (97%)	YP_178269.1
242	334	38.0	a) conserved domain protein	*C. jejuni* RM1221	249/259 (96%)	gb|CP000025.1
			b) conserved domain protein		59/59 (100%)	ZP_01070050.1
255	211	40.3	a) hypothetical protein		134/134 (100%)	gb|CP000025.1
			b) hypothetical protein CJJ26094_0512		44/44 (100%)	ZP_01070032.1
264	290	37.1	a) conserved hypothetical protein		213/213 (100%)	gb|CP000538.1
			b) conserved hypothetical protein		74/82 (90%)	ZP_01071231.1

[a] a) BLASTN hit and b) BLASTX hit

Table 4.13. Unique clones, recovered from suppressive subtractive hybridizations, of *C. jejuni* isolate 8557 (Caco-2 mid invasion) determined to possess significant similarity to *Campylobacter* spp. other than *C. jejuni* 11168 (PMSRU).

Isolate	Percentage of Clones (%) (n=71)
C. jejuni RM1221	20.0%
C. jejuni 81-176	24.0%
C. jejuni HB93-13	1.4%
C. jejuni 43432	4.0%
C. jejuni 11828	1.4%
C. jejuni RM3423	3.0%
C. jejuni 81116	14.0%
C. jejuni RM1167	1.4%
C. jejuni RM224	1.4%

Table 4.14. Isolate 13262 (Caco-2 low invasion) unique clones relative to *C. jejuni* 11168 (PMSRU).

Category & Clone #	Length (bp)	% G+C	BLASTN and BLASTX results [a]	Organism	% Similarity % Identity	Accession no.
Cell Envelop & Surface structures						
1	352	32.0	a) no significant similarities to any nucleic acid			
			b) putative beta-N-acetylgalactosaminyltransferase	*C. jejuni*	83/105 (79%)	ABN41494.1
3	842	33.8	a) co-chaperone protein DnaJ	*C. jejuni* 81-176	211/228 (92%)	CP000538.1
			b) chaperone protein dnaJ	*C. doylei* 269.97	76/90 (84%)	ZP_01070931.1
97	244	42.6	a) cell division protein FtsK, putative	C. *jejuni* 81-176	151/162 (93%)	CP000538.1
			b) dna translocase spoiiie	*C. doylei* 269.97	53/63 (84%)	ZP_01070300.1
100	497	32.1	a) no significant similarities to any nucleic acid			
			b) possible sugar transferase	*C. jejuni* CG8486	113/125 (90%)	ZP_01810450.1
173	375	34.6	a) peptidase family protein	*C. jejuni* 81-176	289/305 (94%)	CP000538.1
			b) flagellar motor switch protein	*C. jejuni* CG8486	63/72 (87%)	ZP_01810543.1
174	251	37.8	a) flagellar biosynthetic protein FlhB	*C. jejuni* 81-176	106/110 (96%)	CP000538.1
			b) ATP synthase F0 sector B subunit	*C. jejuni* CG8486	28/30 (93%)	ZP_01810581.1
181	345	32.7	a) DNA polymerase III, beta subunit	*C. jejuni* Rm1221	290/299 (98%)	gb|CP000025.1
			b) DNA polymerase III, beta subunit	*C. jejuni* Rm1221	97/101 (96%)	ZP_01068757.1
237	553	35.2	a) flagellar hook assembly protein	*C. doylei* 269.97	122/135 (90%)	CP000025.1
			b) ABC transporter, ATP-binding protein	*C. doylei* 269.97	26/27 (96%)	ABS43505.1
Bacterial Toxin						
273	232	41.8	a) CDT operon (*cdtA*, *cdtB*, *cdtC* genes)	*C. lari*	95/109 (87%)	AB292356.1
			b) cytolethal distending toxin C	*C. lari*	31/39 (79%)	dbj|BAF48048.1
Restriction-modification, recombination & repair						
9	429	35.2	a) DNA-binding protein Roi	*C. jejuni* RM1221	192/201 (95%)	CP000025.1
			b) DNA-binding protein Roi	*C. jejuni* RM1221	67/69 (97%)	YP_179421.1
22	351	35.8	a) terminase B protein, putative	*C. jejuni* RM1221	182/185 (98%)	|CP000025.1
			b) terminase B protein, putative	*C. jejuni* RM1221	43/45 (95%)	|CP000025.1

[a] a) BLASTN hit and b) BLASTX hit

Table 4.14 cont. 13262 (Caco-2 low invasion) unique clones relative to *C. jejuni* 11168 (PMSRU).

Category & Clone #	Length (bp)	% G+C	BLASTN and BLASTX results [a]	Organism	% Similarity % Identity	Accession no.
45	321	36.1	a) no significant Similarities to any nucleic acid			
			b) phenylalanyl-tRNA synthetase, beta subunit	*C. jejuni* 260.94	65/83 (78%)	ZP_01070273.1
98	735	35.5	a) type II restriction-modification enzyme	*C. jejuni* RM1221	409/415 (98%)	CP000025.1
			b) type II restriction-modification enzyme	*C. jejuni* RM1221	140/150 (93%)	YP_178058.1
106	230	39.5	a) single-stranded-DNA-specific exonuclease *RecJ*	*C. jejuni* 81-176	151/157 (96%)	CP000538.1
			b) putative single-stranded-DNA-specific exonuclease	*C. jejuni* CG8486	51/54 (94%)	ZP_01810523.1
143	664	41.3	a) rRNA-23S ribosomal RNA	*C. jejuni* RM1221	582/593 (98%)	CP000025.1
			b) conserved hypothetical protein	*C. jejuni* CF93-6	48/51 (94%)	ZP_01067405.1
199	263	33.8	a) possible polysaccharide modification protein		173/187 (92%)	AY332625.1
			b) hypothetical protein Cj8486_1461c		59/63 (93%)	ZP_01810447.1
275	390	38.9	a) DNA-binding protein Roi	*C. doylei 269.97*	263/287 (91%)	CP000768.1
			b) conserved domain protein	*C. jejuni* RM1221	52/58 (89%)	AAW35141.1
Transport						
13	377	38.5	a) trkA domain protein	*C. jejuni* 81-176	250/258 (96%)	CP000538.1
			b) hypothetical protein Cj8486_1071	*C. jejuni* CG8486	86/86 (100%)	ZP_01809767.1
111	265	34.3	a) di-/tripeptide transporter	*C. jejuni* 81-176	184/189 (97%)	gb|CP000538.1
			b) di- and tri-peptide transporter	*C. doylei* 269.97	43/45 (95%)	AAV30680.1
129	492	34.3	a) CTP synthase	*C. jejuni* 81-176	99/99 (100%)	CP000538.1
			b) CTP synthase	*C. jejuni* 81-176	99/99 (100%)	
190	145	37.2	a) putative peptide ABC-transport system periplasmic	*C. jejuni* 81-176	86/87 (98%)	DQ493924.1
			b) anaerobic dimethyl sulfoxide reductase chain A	*C. jejuni* 81-176	28/29 (97%)	AAY53798.1
191	412	33.4	a) GlnD family protein	*C. jejuni* RM1221	258/261 (98%)	CP000025.1
			b) GlnD family protein	*C. jejuni* RM1221	85/88 (96%)	YP_179542.1

[a] a) BLASTN hit and b) BLASTX hit

Table 4.14 cont. 13262 (Caco-2 low invasion) unique clones relative to *C. jejuni* 11168 (PMSRU).

Category & Clone #	Length (bp)	% G+C	BLASTN and BLASTX results [a]	Organism	% Similarity % Identity	Accession no.
213	511	30.2	a) no significant similarities to any nucleic acid			
			b) ABC transporter	*Beggiatoa* sp. PS	45/146 (30%)	EDN71435.1
279	269	37.2	a) macrolide-specific efflux protein macA	*C. jejuni* 81-176	209/213 (98%)	CP000538.1
			b) macrolide-specific efflux protein macA	*C. jejuni* 81-176	71/71 (100%)	YP_001398157.1
Other (bacteriophage sequence)						
125	406	30.8	a) site-specific recombinase, phage integrase family	*C. jejuni* RM1221	328/330 (99%)	CP000025.1
			b) site-specific recombinase, phage integrase family	*C. jejuni* RM1221	98/100 (98%)	YP_178560.1
Small molecular metabolism						
2	438	32.4	a) HAD-superfamily phosphatase, subfamily IIIC	*C. jejuni* 81-176	90/90 (100%)	CP000538.1
			b) hypothetical protein Cjejd_02000147	*C. doylei* 269.97	71/72 (92%)	ZP_01807491.1
16	796	31.4	a) D12 class N6 adenine-specific DNA methyltransferase	*C. jejuni* RM1221	274/279 (98%)	CP000025.1
			b) D12 class N6 adenine-specific DNA methyltransferase	*C. jejuni*. CG8486	118/153 (77%)	ZP_01810087.1
30	251	43.8	a) carbamoyl-phosphate synthase, large subunit	*C. jejuni* 81-176	99/105 (94%)	CP000538.1
			b) carbamoyl-phosphate synthase large chain	*C. jejuni* CG8486	33/34 (97%)	ZP_01810658.1
55	145	47.5	a) anaerobic dimethyl sulfoxide reductase chain A	*C. jejuni* 81-176	83/84 (98%)	CP000538.1
			b) anaerobic dimethyl sulfoxide reductase chain A	*C. jejuni*	27/27 (100%)	AAY53798.1
59	244	45.9	a) polyphosphate kinase	*C. jejuni* RM1221	144/148 (98%)	CP000025.1
			b) conserved hypothetical protein	*C. jejuni* 260.94	44/44 (100%)	ZP_01069225.1
115	194	40.7	a) 3-dehydroquinate synthase	*C. jejuni* 81-176	111/117 (94%)	CP000538.1
			b) 3-dehydroquinate synthase	*C. doylei* 269.97	36/40 (90%)	ZP_01069050.1

[a] a) BLASTN hit and b) BLASTX hit

Table 4.14 cont. 13262 (Caco-2 low invasion) unique clones relative to *C. jejuni* 11168 (PMSRU).

Category & Clone #	Length (bp)	% G+C	BLASTN and BLASTX results [a]	Organism	% Similarity % Identity	Accession no.
140	109	37.6	a) anaerobic dimethyl sulfoxide reductase chain A	*C. jejuni* 81-176	54/54 (100%)	CP000538.1
			b) anaerobic dimethyl sulfoxide reductase chain A	*C. jejuni*	18/19 (94%)	AAY53798.1
179	354	35.6	a) no significant similarities to any nucleic acid			
			b) GDP-L-fucose synthetase co-enzyme binding	*C. jejuni* 84-25	70/102 (68%)	ZP_01099795.1
187	599	38.8	a) dihydroorotase, homodimeric type D12 class N6 adenine-specific DNA methyltransferase	*C. jejuni* RM1221	376/409 (91%)	gb|CP000025.1
			b) dihydroorotase	*C. jejuni* RM1221	101/107 (94%)	YP_178329.1
236	323	39.6	a) no significant similarities to any nucleic acid			
			b) biotin sulfoxide reductase (*bisC*)	*C. lari* RM2100	74/82 (90%)	ZP_00368912.1
245	238	36.1	a) hydrogenase, (NiFe)/(NiFeSe) small subunit family	*C. jejuni* RM1221	157/192 (96%)	CP000025.1
			b) hydrogenase, (NiFe)/(NiFeSe) small subunit family	*C. jejuni* RM1221	51/54 (94%)	ZP_01070165.1
277	269	36.1	a) hydrogenase assembly chaperone HypC/HupF	*C. jejuni* 81-176	140/152 (92%)	CP000538.1
			b) hydrogenase assembly chaperone HypC/HupF	*C. coli* RM2228	51/61 (83%)	ZP_00366792.1
Hypothetical & unknown						
31	117	58.0	a) no significant similarities to any nucleic acid			
			b) no significant similarities to any protein			
60	75	50.1	a) no significant similarities to any nucleic acid			
			b) no significant similarities to any protein			

[a] a) BLASTN hit and b) BLASTX hit

Table 4.14 cont. 13262 (Caco-2 low invasion) unique clones relative to *C. jejuni* 11168 (PMSRU).

Category & Clone #	Length (bp)	% G+C	BLASTN and BLASTX results [a]	Organism	% Similarity % Identity	Accession no.
68	597	30.0	a) no significant similarities to any nucleic acid			
			b) possible sugar transferase	*C. jejuni* CG8486	159/166 (95%)	ZP_01810450.1
69	85	57.6	a) uncultured bacterium gene for 16S rRNA		44/44 (100%)	AB177205.1
			b) no similarity to any protein			
124	378	30.9	a) hypothetical protein	*C. jejuni* RM1221	170/172 (98%)	CP000025.1
			b) hypothetical protein CJE0592	*C. jejuni* RM1221	38/40 (95%)	YP_178608.1
136	430	35.1	a) polyribonucleotide nucleotidyltransferse, conserved	*C. jejuni* RM1221	347/353 (98%)	CP000025.1
			hypothetical protein,	*C. jejuni* 260.94	96/98 (97%)	ZP_01069129.1
			b) conserved hypothetical protein			
146	200	43.5	a) hypothetical protein	*C. jejuni* CF93-6	121/123 (98%)	ZP_01068327.1
			b) hypothetical protein	*C. jejuni* CF93-6	40/41 (97%)	ZP_01068327.1
150	297	34.3	a) conserved hypothetical protein	*C. jejuni* 81-176	211/221 (95%)	CP000538.1
			b) hypothetical protein CJJ81176_0772,	*C. jejuni* 81-176	19/19 (100%)	YP_001000440.1
175	383	32.6	a) no significant similarities to any nucleic acid			
			b) hypothetical protein	*C. jejuni* RM1221	45/106 (45%)	ZP_01834321.1
192	83	48.2	a) no significant similarities to any nucleic acid			
			b) no significant similarities to protein			
201	498	32.5	a) conserved domain protein	*C. jejuni* RM1221	396/399 (99%)	CP000025.1
			b) conserved domain protein	*C. jejuni* RM1221	132/136 (97%)	AAW35931.1
211	250	37.2	a) conserved domain protein	*C. jejuni* RM1221	173/174 (99%)	CP000025.1
			b) hypothetical protein CJE0556	*C. jejuni* RM1221	59/64 (92%)	YP_178572.1
218	166	51.2	a) hypothetical protein	*C. jejuni* RM1221	187/189 (98%)	P000025.1
			b) hypothetical protein CJE0590	*C. jejuni* RM1221	62/63 (98%)	YP_178606.1
222	321	38.6	a) hypothetical protein	*C. jejuni*	227/245 (91%)	CP000025.1
			b) hypothetical protein CJJ26094_1412	*C. jejuni* 260.94	69/80 (86%)	ZP_01069043.1
240	248	38.7	a) no significant similarities to any nucleic acid			
			b) no significant similarities to any protein			

[a] a) BLASTN hit and b) BLASTX hit

Table 4.14 cont. 13262 (Caco-2 low invasion) unique clones relative to *C. jejuni* 11168 (PMSRU).

Category & Clone #	Length (bp)	% G+C	BLASTN and BLASTX results [a]	Organism	% Similarity % Identity	Accession no.
253	289	35.6	a) hypothetical protein	*C. doylei* 269.97	205/213 (96%)	CP000768.1
			b) hypothetical protein JJD26997_1925	*C. doylei* 269.97	63/64 (98%)	YP_001398856.1
271	401	31.6	a) hypothetical protein	*C. jejuni* RM1221	219/226 (96%)	CP000025.1
			b) conserved hypothetical protein	*C. jejuni* RM1221	60/81 (74%)	ABS43420.1

[a] a) BLASTN hit and b) BLASTX hit

Table 4.15. Unique clones, recovered from suppressive subtractive hybridizations, of *C. jejuni* isolate 13262 (Caco-2 low invasion) determined to possess significant similarity to *Campylobacter* spp. other than *C. jejuni* 11168 (PMSRU).

Isolate	Percentage of Clones (n=59)
C. jejuni 81-176	31.0%
C. jejuni	2.0%
C. jejuni CH8486	4.0%
C. jejuni RM1221	27.0%
C. jejuni 260.94	2.0%
C. jejuni HS:23	2.0%
C. jejuni 84-25	2.0%
C. jejuni CF93-6	2.0%

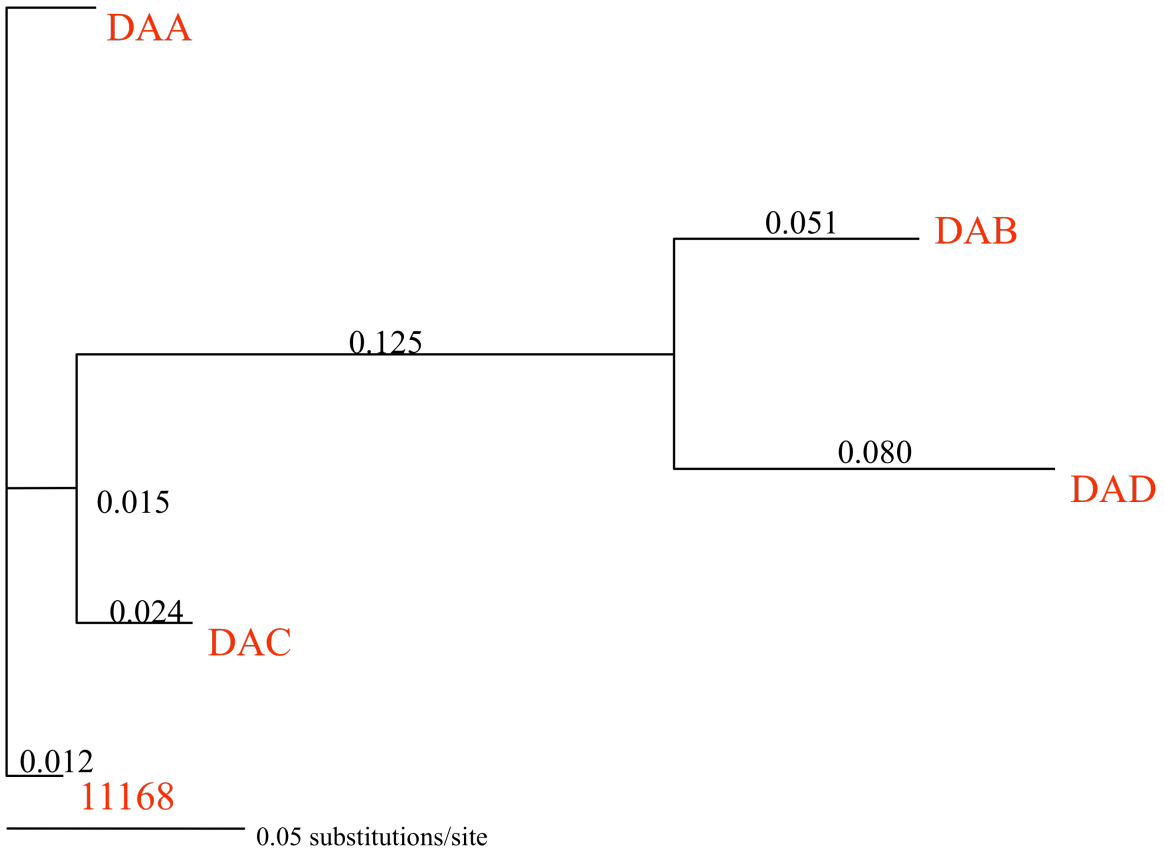

Figure 4.1: Dendrogram of representative *Campylobacter* spp. *flaA* SVR alleles recovered during the Icelandic Epidemiology Investigation.

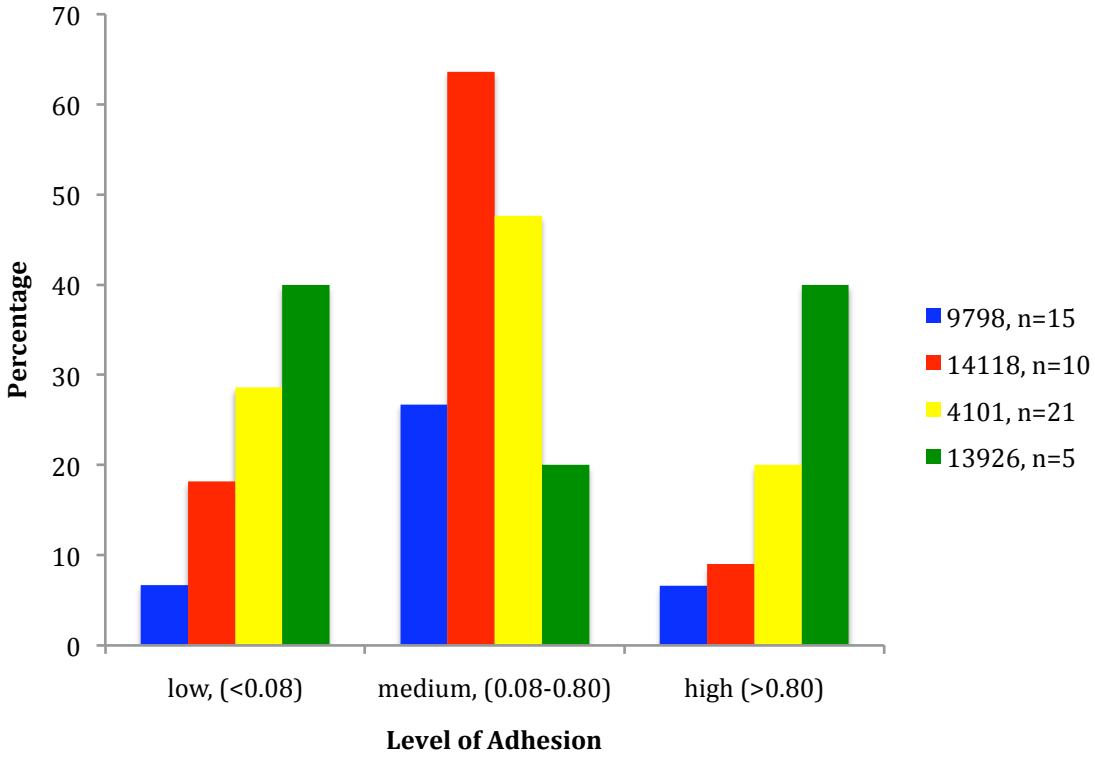

Figure 4.2. Distribution of *Campylobacter* spp. *flaA* SVR allele groups by percent adhesion to Caco-2 cells.

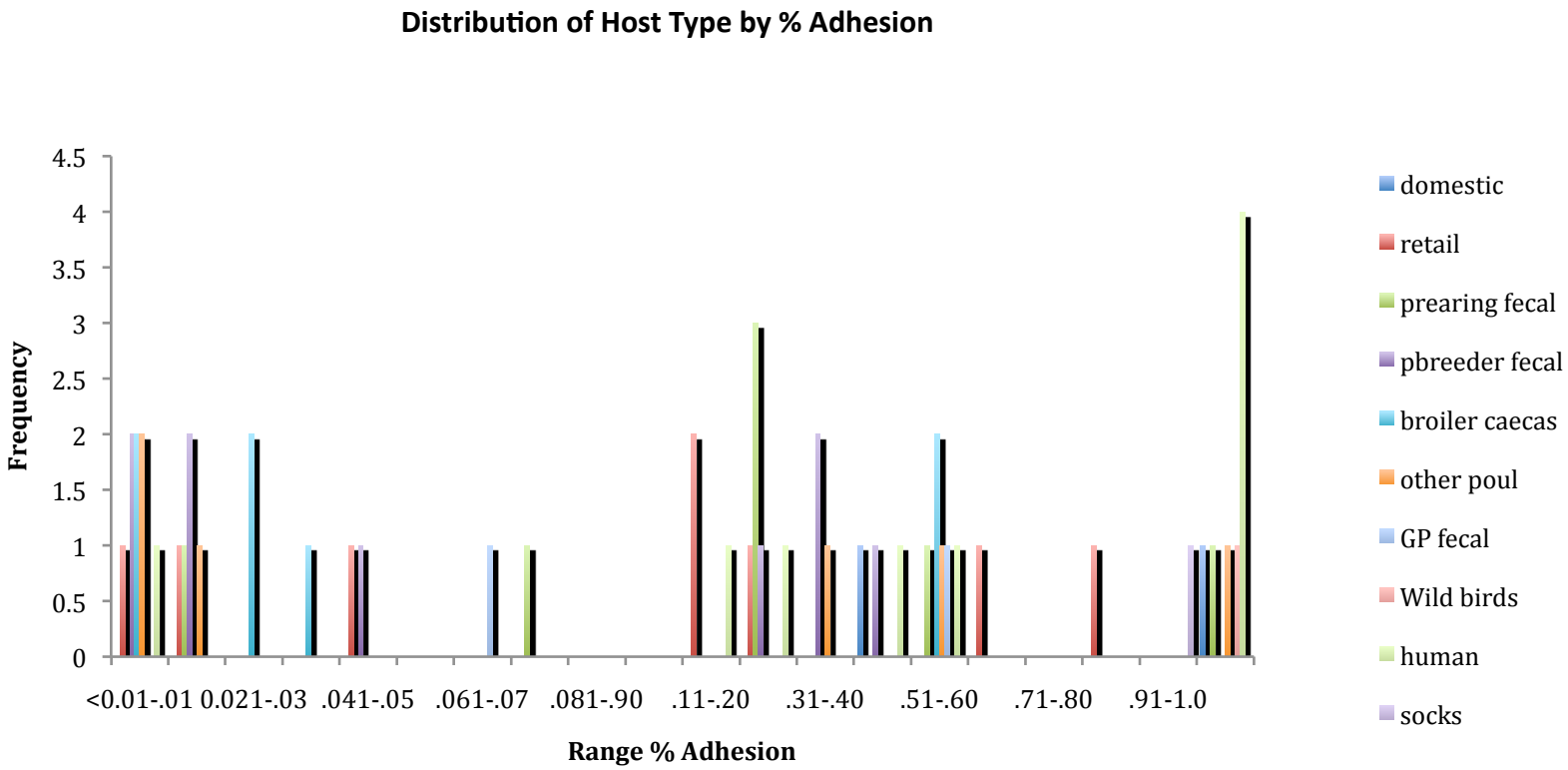

Figure 4.3 Distribution of *Campylobacter* spp. percent adhesion of Caco-2 cells relative to original host.

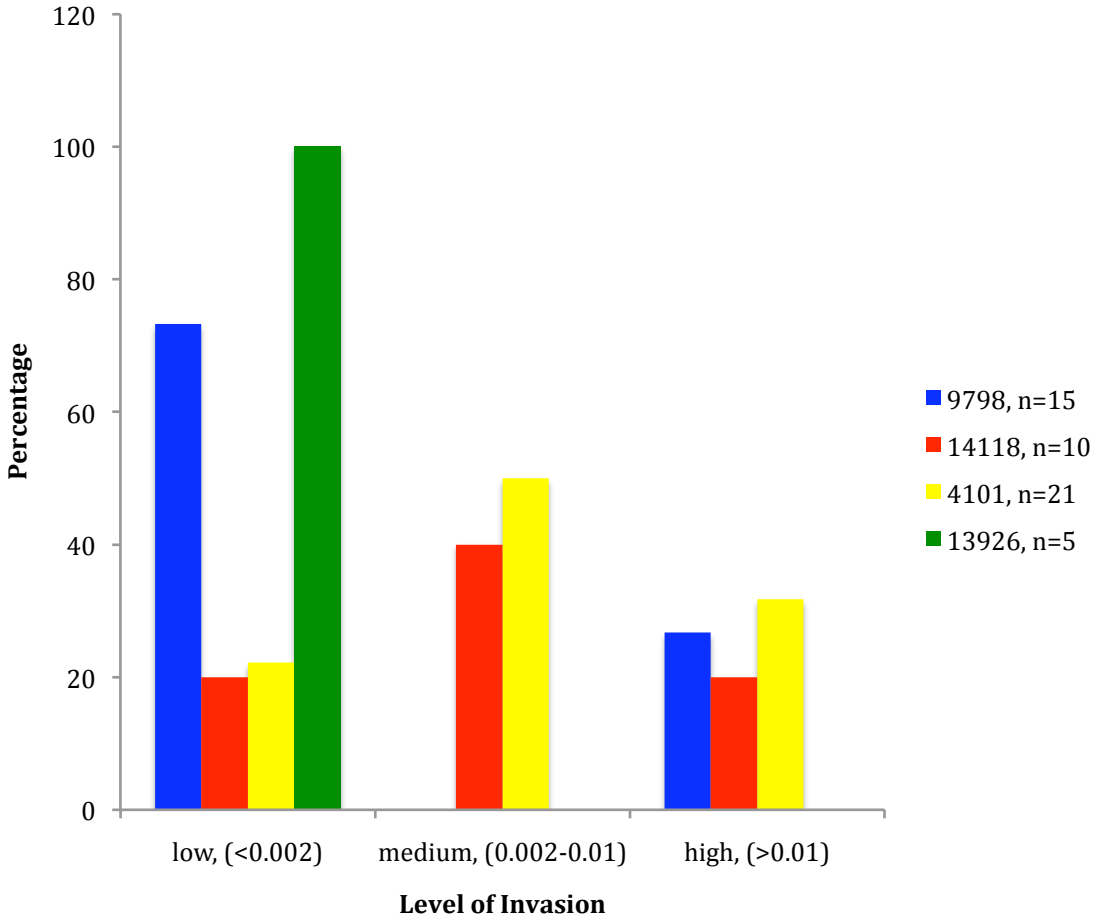

Figure 4.4. Distribution of *Campylobacter* spp. *flaA* SVR allele groups by percent invasion of Caco-2 cells.

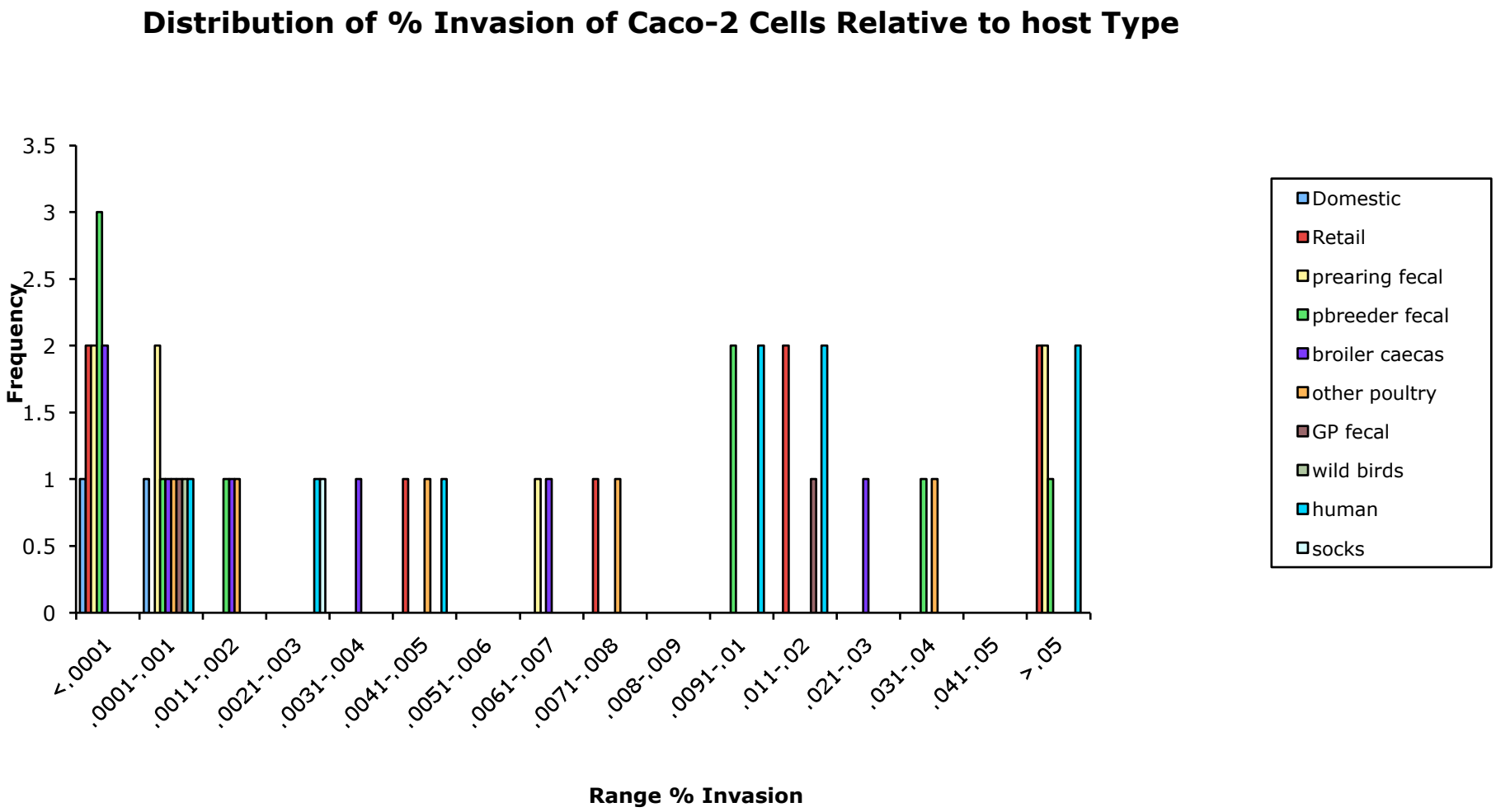

Figure 4.5. Distribution of *Campylobacter* spp. host type by percent invasion of Caco-2 cells.

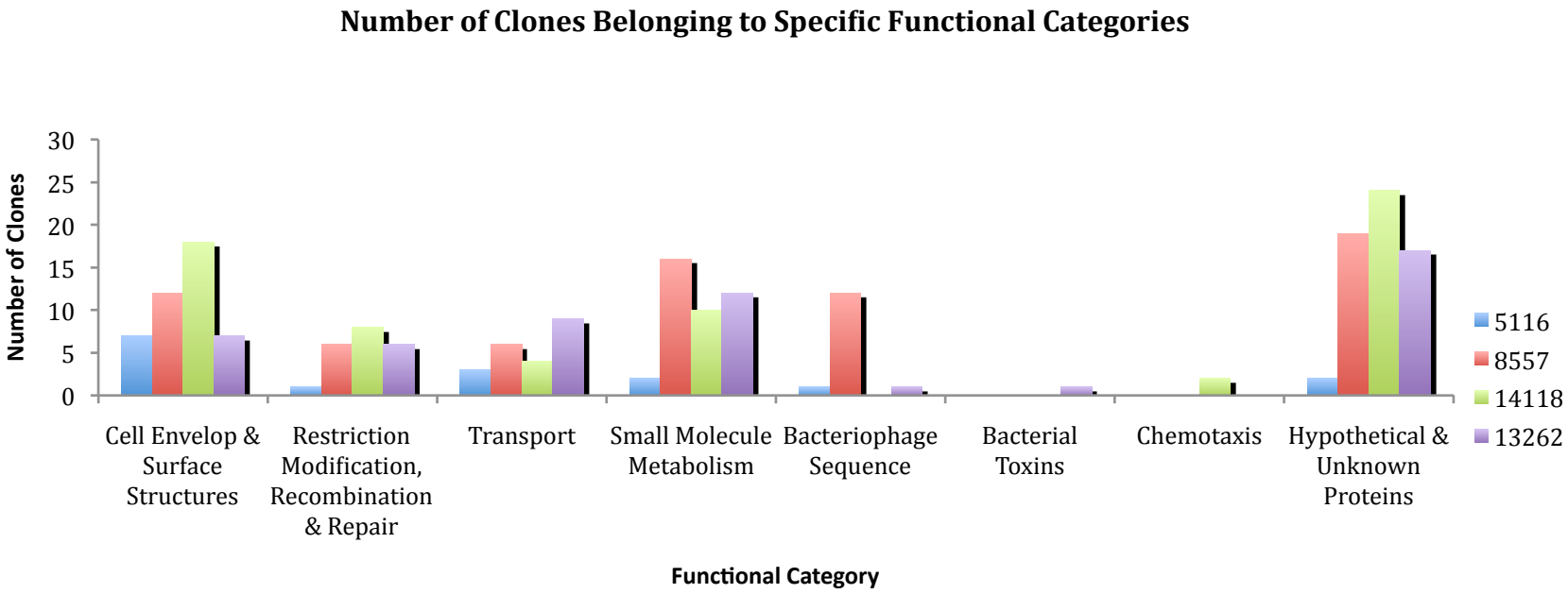

Figure 4.6. Functional categories of clones recovered using suppressive subtractive hybridization on all 4 *C. jejuni* isolates.

Chapter 5

Summary and Conclusions

The studies presented were based on three primary objectives: 1) To optimize conditions for use with the Bactometer® such that efficient and reproducible monitoring of *Campylobacter* spp. for determination of growth curves was achieved in a simple medium, 2) To investigate the adherence and invasiveness of 52 *Campylobacter* spp. isolates using Caco-2 cells, and 3) To investigate the genetic diversity of four *C. jejuni* isolates that demonstrated a wide range of invasiveness towards human colonic cells.

For the first objective, conditions for use with the Bactometer® were investigated to determine efficient and reproducible monitoring of Campylobacter spp. growth curves in a simple medium. Results suggested that isolates be grown on Mueller Hinton plates under a microaerobic atmosphere (37°C; 24 h), followed by transfer to Mueller Hinton biphasic cultures for 6 h (37°C; microaerobic atmosphere). Serial dilutions were inoculated in Bactometer® wells containing 1 mL Mueller Hinton broth plus 0.1M sodium pyruvate. Utilizing the Bactometer® is important since determining growth curves by hand is a time consuming and labor intensive process.

For the second objective, adhesion and invasion assays were performed on 52 *Campylobacter* spp. isolates using human Caco-2 cells. *Campylobacter* spp. exhibited a wide distribution of adhesion and invasion ability, which was determined unrelated to *flaA* SVR allele type. There also appeared to be no relationship between host of recovery source and level of adhesion or invasion. This objective was important in determining if the *flaA SVR* allele type could predict potential virulence.

The last objective investigated the genetic diversity of four *C. jejuni* isolate that demonstrated a wide range of invasiveness towards human colonic cells. Four isolates comprised of the most invasive isolate (14118), the least invasive isolate (13262), and two

isolates in between were selected for DNA: DNA microarray hybridizations and suppressive subtractive hybridizations. DNA:DNA microarray hybridizations identified genes absent relative to 11168 (PMSRU) and also determined 372 genes present in *C. jejuni* isolates 14118, 5116, 8557, and 13262 as well as *C. jejuni* 11168 (PMSRU). Suppressive subtractive hybridizations identified genes absent from *C. jejuni* 11168 (PMSRU). *C. jejuni* 14118 contained a gene from *C. doylei* 269.97 that encoded for a motility accessory factor and a gene involving transport. *C. jejuni* 13262 contained a CDT operon from *C. lari* as well as included a type II restriction system. These results provide further insight into the genetic variability of *Campylobacter* spp. The results facilitated determination of the core *C. jejuni* genome, and also provides information regarding putative virulence factors.

These experiments will hopefully serve as a basis for future investigations, which will help us better understand *Campylobacter* spp. Further studies should investigate genes that code for hypothetical proteins. Understanding *Campylobacter* spp. will potentially help develop better strategies for reducing poultry associated *Campylobacter* spp. and potentially reduce human exposure to the organism so that public health is improved.