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Characterization of *Campylobacter jejuni* Biofilms under Defined Growth Conditions[▽]

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***Campylobacter jejuni* is a major cause of human diarrheal disease in many industrialized countries and is a source of public health and economic burden. *C. jejuni*, present as normal flora in the intestinal tract of commercial broiler chickens and other livestock, is probably the main source of human infections. The presence of *C. jejuni* in biofilms found in animal production watering systems may play a role in the colonization of these animals. We have determined that *C. jejuni* can form biofilms on a variety of abiotic surfaces commonly used in watering systems, such as acrylonitrile butadiene styrene and polyvinyl chloride plastics. Furthermore, *C. jejuni* biofilm formation was inhibited by growth in nutrient-rich media or high osmolarity, and thermophilic and microaerophilic conditions enhanced biofilm formation. Thus, nutritional and environmental conditions affect the formation of *C. jejuni* biofilms. Both flagella and quorum sensing appear to be required for maximal biofilm formation, as *C. jejuni flaAB* and *luxS* mutants were significantly reduced in their ability to form biofilms compared to the wild-type strain.**

Campylobacter jejuni is a gram-negative, curved-to-spiral rod with polar flagella and grows best in a microaerophilic environment ranging from 37°C to 42°C (5, 8, 15, 29, 32). In the United States, it is estimated that approximately 2.1 to 2.4 million cases of campylobacteriosis occur annually, with a cost of $8 billion (20, 21).

Symptomatic infections of campylobacteriosis may consist of an acute onset of watery diarrhea, abdominal pain, fever, and the presence of blood and leukocytes in the stools. The disease is usually self-limiting, lasting from 2 to 11 days (5, 7, 20). Long-term secondary effects of infection may include reactive arthritis, Reiter's syndrome, and Guillain-Barré syndrome (19, 23).

Campylobacter spp. are considered normal flora of the gastrointestinal tract of a number of domestic animals and birds, such as commercial broiler chickens (1, 2, 6, 9, 38). *Campylobacter* spp. shed by these birds can enter waterways, which in turn can act as a source of contamination for other animals. *Campylobacter* infections occur through oral routes, including ingestion of contaminated water, unpasteurized milk, and undercooked or raw foods, such as poultry (6, 9, 38). However, consumption of raw milk and undercooked poultry is considered the major source of *Campylobacter* infections.

In most settings, natural, industrial, or clinical, bacteria are usually found in biofilms rather than in the planktonic state seen in the laboratory (7, 24). Current theories suggest that transition to a biofilm state is dependent on the nutritional content of the surrounding medium. Previous research has demonstrated that biofilm formation takes place via multiple steps, and upon completion, a mature, dynamic, three-dimensional structure is formed (11, 16, 24, 26). Some of the current biofilm models indicate that the participation of flagella and pili is important in the growth of the microcolony, especially during the early stages of biofilm formation (3, 13, 16, 17, 24, 27, 31).

C. jejuni has the ability to form biofilms in the watering supplies and plumbing systems of animal husbandry facilities and animal-processing plants, and these biofilms may provide a continual inoculum for domesticated animals and lead to human infections (9, 38). However, this possibility is supported by a very limited number of studies showing that *C. jejuni* can form biofilms on abiotic surfaces.

In this study, a biofilm assay was used to investigate the mechanisms required for *C. jejuni* biofilm formation on abiotic surfaces. Various environmental factors, including temperature, oxygen tension, and various nutritional factors of the bathing media, can influence the ability of *C. jejuni* to form biofilms on abiotic surfaces. In addition, both flagella and quorum sensing appear to affect biofilm formation in this organism.

MATERIALS AND METHODS

Bacterial strains and media. All *C. jejuni* isolates (Table 1) were maintained on Mueller-Hinton agar (Difco) supplemented with 5% bovine blood at 37°C in a 10% CO_2 incubator.

Biofilm assay. *C. jejuni* isolates were grown overnight in Mueller-Hinton broth (MHB; Difco) at 37°C and 10% CO_2 with shaking to an optical density at 600 nm (OD_{600}) of 0.25. The wells of 24-well polystyrene plates (Corning) containing 1 ml MHB or brucella (Difco) or Bolton (Difco) broth were inoculated with overnight cultures of *C. jejuni* isolates to an OD_{600} of 0.025 (~2.5×10^7 CFU). Plates were incubated at 37°C or 25°C in a 10% CO_2 atmosphere or aerobically for 24, 48, or 72 h. Following incubation, the medium was removed, the wells were dried for 30 min at 55°C, and 1 ml 0.1% crystal violet (CV) was added for 5 min at room temperature. The unbound CV was removed, and the wells were washed twice with H_2O. The wells were dried at 55°C for 15 min, and bound CV was decolorized with 80% ethanol-20% acetone. One hundred microliters of this solution was removed from the wells and placed in a 96-well plate, and the absorbance at 570 nm (A_{570}) was determined using a microplate reader (Bio-Tek) to determine biofilm formation.

In order to determine the effects of osmolytes, they were added to MHB prior

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TABLE 1. *C. jejuni* isolates used in this study

Strain	Characteristics	Reference or source
F38011	Human clinical isolate	39
M129	Human clinical isolate	12
NCTC11168	Type strain; genome sequence has been determined	28
S2B	Chicken normal flora isolate	L. A. Joens, unpublished data
UMC3	Human clinical isolate	University of Arizona Medical Center, Tucson, AZ

to the biofilm assay. Plates were incubated at 37°C in a 10% CO_2 atmosphere for 24 h.

***C. jejuni* biofilm formation on abiotic surfaces.** Sterile, ~1- by 4-cm coupons of acrylonitrile butadiene styrene plastic (ABS), polyvinyl chloride plastic (PVC), polystyrene, or copper were placed in 15-ml polypropylene tubes with 5 ml MHB such that the coupon was completely submerged. The tubes were inoculated with *C. jejuni* to an OD_{600} of 0.025 and incubated for 24 h at 37°C and 10% CO_2. The coupons were aseptically removed and placed in sterile 15-ml tubes with 2 ml of 0.1 M phosphate-buffered saline, pH 7.3, and 20- by 4-mm sterile glass beads. The bacteria were detached by vortex mixing on full speed for 1 min, which did not affect cell viability (data not shown). Viable bacteria were enumerated by dilution plating on Mueller-Hinton agar supplemented with 5% blood.

Inhibition of protein synthesis. Overnight cultures of *C. jejuni* in MHB were treated with 0.5 μg/ml chloramphenicol (Cm) for 15 min at room temperature prior to being assayed for biofilm formation in the absence of antibiotic. At this concentration of Cm, protein synthesis was inhibited, but the viability of the *C. jejuni* isolates was not impaired (data not shown). Biofilm formation of Cm-treated *C. jejuni* isolates was assayed as described above.

Culture supernatant fluid. Bacterial culture supernatant fluids (CSFs) were collected after 24 h of growth in the appropriate culture medium (Table 2). Cells were removed by centrifugation at 5,000 × *g*, and the CSF was filtered through a 0.22-μm filter. CSF or uninoculated culture medium was mixed 1:1 with MHB, and the biofilm formation of *C. jejuni* isolates was assayed in these media as described above.

Construction of *C. jejuni flaAB* and *luxS* mutants. *C. jejuni* mutants with mutations in genes encoding flagella or quorum-sensing molecule autoinducer 2 (AI-2) were constructed to assess the role of these factors in biofilm formation. The *C. jejuni* M129 *flaA flaB* double mutant was constructed as follows. PCR was used to amplify a 0.9-kb fragment containing the 5′ end of *flaA* from *C. jejuni* M129 genomic DNA with the primers 5′-CTTTGGCTATCTCGAGACAGGC ACTC-3′ and 5′-AAGCTGCAAGCTTGGTGTTAATACGA-3′, while a 0.9-kb fragment containing the 3′ end of *flaB* was amplified with the primers 5′-AAA TCAGAGAATTCATTGGTTCGGTG-3′ and 5′-TAACAACAGGATCCTCA TAGGTCAGG-3′. The resulting products were digested with XhoI-HindIII and EcoRI-BamHI, respectively (restriction sites are underlined in the primer sequences). The two fragments were cloned consecutively into the vector pSJB21, which contains a *Campylobacter coli aphA-3* kanamycin resistance gene cloned into the HindIII and EcoRI sites of pBC KS, such that the fragments flanked the resistance gene and were oriented in the same direction. This allelic exchange plasmid was introduced into *C. jejuni* strain M129 by electroporation, and putative mutants were selected on Mueller-Hinton agar supplemented with 5% blood and 50 μg/ml kanamycin. Allelic replacement was confirmed by PCR and Southern blotting. As *flaA* and *flaB* are adjacent in *C. jejuni*, the resulting *flaAB* mutant contained only the 5′ end of *flaA* and the 3′ end of *flaB*, disrupting both genes.

The *C. jejuni luxS* mutant was constructed similarly, by cloning PCR products derived from the 5′ end of the *C. jejuni* M129 *luxS* gene, which was amplified with the primers 5′-CTTCTTGTAACTCGAGTTGTCGTATC-3′ and 5′-AATCAA ATAAGCTTATATCATCACCC-3′, and those from the 3′ end of *luxS*, which was amplified with primers 5′-GAACTTAAGAATTCCCAATGCGGAAC-3′ and 5′-ATCTTTATGGGATCCTACGCCTTGAG-3′, into pSJB21. The resulting plasmid was then used for allelic exchange as described above.

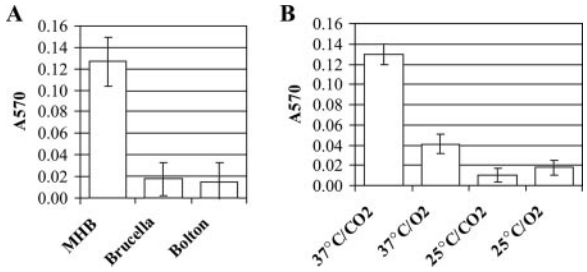

FIG. 1. Effects of growth medium, temperature, and oxygen tension on *C. jejuni* biofilm formation. (A) *C. jejuni* M129 biofilm formation in MHB and brucella and Bolton broths at 37°C and 10% CO_2. (B) *C. jejuni* M129 biofilm formation in MHB in 10% CO_2 or under aerobic conditions at either 37°C or 25°C. Biofilm formation was assessed by CV staining. Experiments were performed in triplicate on three separate occasions, and error bars represent one standard deviation from the mean.

RESULTS

Environmental factors affect biofilm formation. MHB and brucella and Bolton broths were assessed for their ability to promote *C. jejuni* M129 biofilm formation. The more nutrient-rich brucella and Bolton broths did not support biofilm formation, while significant biofilm formation occurred in the less nutrient-rich MHB (Fig. 1A).

C. jejuni biofilm formation was also assayed under different temperatures, oxygenation, and/or osmolyte (glucose, sucrose, or NaCl) concentration. As anticipated, oxygen tension and temperature had significant effects on the biofilm formation, and biofilm formation was enhanced under conditions which favored the growth of *C. jejuni* (Fig. 1B). Furthermore, any concentration of glucose, sucrose, or NaCl added to the MHB resulted in significantly decreased biofilm formation (Fig. 2). During light microscopy examination of *C. jejuni* isolates exposed to these osmolytes, cells were observed transitioning from spiral-shaped rods to a coccoid form (data not shown). These results indicate that environmental conditions and the nutritional state of the medium can influence the ability of *C. jejuni* to form biofilms.

Biofilm formation on abiotic surfaces. The ability of *C. jejuni* to form biofilms on materials used for commercial water pipes is of particular interest. *C. jejuni* M129 formed biofilms to various degrees on the hydrophilic materials glass and copper

TABLE 2. Bacterial and culture conditions used for production of CSF

Bacterial species	Strain	Culture conditions	Reference or source
Arcanobacterium pyogenes	BBR1	Trypticase soy broth (TSB; Difco) supplemented with 5% newborn calf serum (Omega Scientific), 37°C, aerobic	4
Clostridium perfringens	Strain 13	TSB supplemented with 0.5% yeast extract (Difco) and 0.05% cysteine, 37°C, anaerobic	35
Pseudomonas aeruginosa	9027	MHB, 37°C, aerobic	ATCC
Pseudomonas fluorescens	PF-5	MHB, 37°C, aerobic	10
Chromobacterium violaceum	CV206	MHB, 37°C, aerobic	37

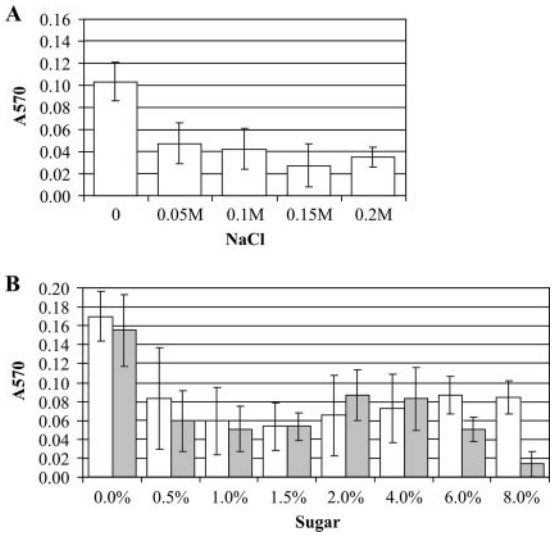

FIG. 2. Effect of NaCl (A) or sucrose or glucose (B) on the ability of *C. jejuni* M129 to form biofilms, as measured by CV staining. (B) White bars represent sucrose, and gray bars represent glucose. Experiments were performed three times in triplicate, and error bars represent one standard deviation from the mean.

and the hydrophobic plastics polystyrene, polypropylene, polycarbonate, ABS, and PVC (data not shown). Differences in the densities of adherent bacteria were observed during microscopy. To further study the ability of *C. jejuni* to form biofilms on these surfaces, materials commonly used in watering systems, such as ABS, PVC, and copper, and a control material, polystyrene, were quantitatively assayed for the promotion of biofilm formation. *C. jejuni* more readily attached to and formed biofilms on hydrophobic surfaces, but to various degrees (Fig. 3). However, *C. jejuni* showed a relative reduction in biofilm formation on the hydrophilic material copper, which may be a result of copper toxicity (Fig. 3).

Protein synthesis is required for biofilm formation. Gene expression studies indicate that there is a difference in the profiles of proteins synthesized by planktonic versus biofilm-grown cells, suggesting that the synthesis of certain proteins may be required for biofilm formation (11, 13, 24). Protein synthesis inhibitors can markedly decrease biofilm formation and cause the release of attached bacteria from a biofilm.

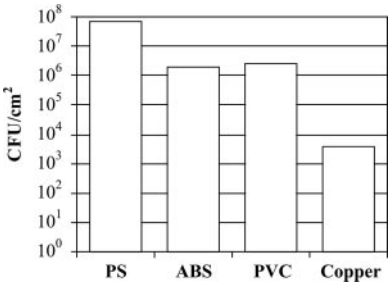

FIG. 3. Biofilm formation of *C. jejuni* M129 on coupons of polystyrene (PS), ABS, PVC, or copper was measured by a viable count following 24 h of incubation at 37°C in 10% CO_2. Experiments were performed three times in triplicate, and the graph depicts data from a representative experiment.

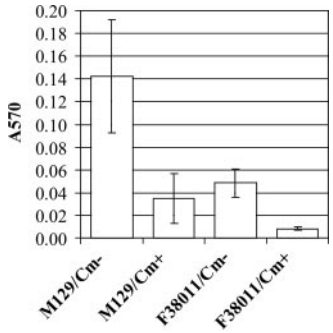

FIG. 4. Cm inhibits biofilm formation of *C. jejuni*. *C. jejuni* strains M129 and F38011 were treated with 0.5 μg/ml Cm for 15 min or left untreated prior to a standard biofilm assay in medium without antibiotic. Experiments were performed three times in triplicate, and error bars represent one standard deviation from the mean.

When *C. jejuni* cells were pretreated with Cm (0.5 μg/ml), a significant reduction in biofilm formation was observed compared to what was observed with untreated control cultures (Fig. 4). These results suggest that *C. jejuni* may synthesize proteins required for attachment and biofilm formation in response to appropriate signals and/or growth conditions.

Flagella play a role in *C. jejuni* biofilm formation. Flagella have been shown to have a role in biofilm formation and affect the rate of attachment by overcoming repulsive forces associated with the surface (15, 17, 26, 27). In this study, a *C. jejuni* flagellum-deficient mutant (M129::*flaAB*) was constructed and assayed for its ability to form biofilms. The M129::*flaAB* mutant grew at the same rate and to the same final OD as wild-type M129 (data not shown). However, this mutant showed a slight reduction in biofilm formation compared to that of the wild type at 24 h, but at 48- and 72-h time points, biofilm formation was markedly decreased (Fig. 5A). The abilities of the *flaAB* mutant and wild-type M129 to attach to polystyrene surfaces were also observed directly using light microscopy (Fig. 5B). An increase of adherent cells over 72 h was observed for wild-type M129. However, with the *flaAB* mutant, very few cells were adherent to the surface after 24 h compared to what was observed with the wild type. These data indicate the importance of flagella in the formation of the mature *C. jejuni* biofilm.

Quorum-sensing signals influence biofilm formation. To assay the possibility that quorum sensing plays a role in *C. jejuni* biofilms, a *luxS* mutant deficient in the production of the quorum-sensing signaling molecule AI-2 (14) was constructed. AI-2 played a significant role in *C. jejuni* biofilm development, as at 48- and 72-h time points, there was a reduction in biofilm formation for the *luxS* mutant compared with that for the wild-type *C. jejuni* strain M129 (Fig. 5A). To confirm this observation, the *luxS* mutant was grown in the presence of CSF from wild-type M129 mixed 1:1 with MHB. CSF was obtained from cultures of *C. jejuni* M129 grown under conditions favorable to the production of quorum-sensing molecules. In the presence of wild-type M129 CSF, an increase in *luxS* mutant biofilm formation was observed (Fig. 5C). The *luxS* mutant was not defective for growth compared to the wild type (data not shown).

Few environmental biofilms contain a single bacterial spe-

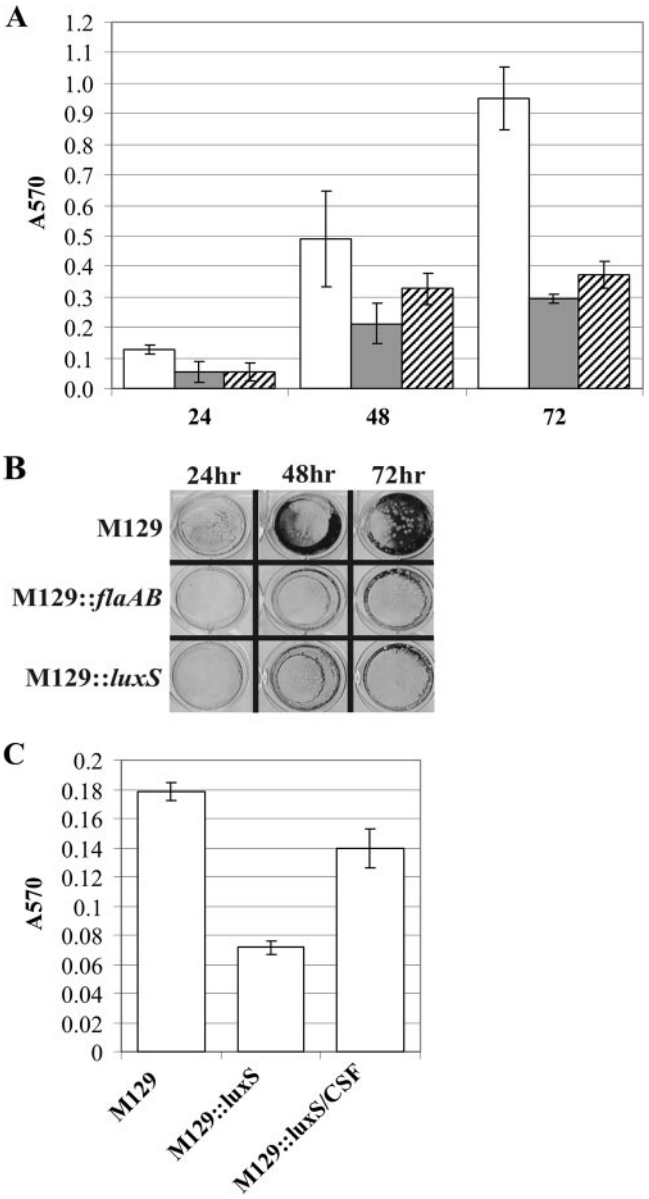

FIG. 5. Flagella and quorum sensing positively influence biofilm formation in *C. jejuni*. *C. jejuni* M129, M129::*flaAB*, and M129::*luxS* strains were subjected to a standard biofilm assay. (A) Biofilm formation at 24, 48, and 72 h postinoculation was measured by CV staining. White bars represent M129, gray bars represent the M129::*flaAB* mutant, and hatched bars represent the M129::*luxS* mutant. (B) Representative wells at 24, 48, and 72 h postinoculation showing CV staining prior to decolorization. (C) CSF from wild-type M129 rescued biofilm formation in the M129::*luxS* mutant. Wild-type M129 CSF was mixed 1:1 with MHB and was used as the growth medium in a standard biofilm assay. Experiments were performed three times in triplicate, and error bars represent one standard deviation from the mean.

cies, and the structure of a biofilm, with cells in close proximity to one another, lends itself to interspecies signaling (13, 36). During this study, CSFs were prepared from various gram-negative and gram-positive bacteria grown under conditions favorable for the expression of quorum-sensing molecules. *C. jejuni* isolate M129 was grown in the presence of these CSFs mixed 1:1 with MHB, which was required to support the

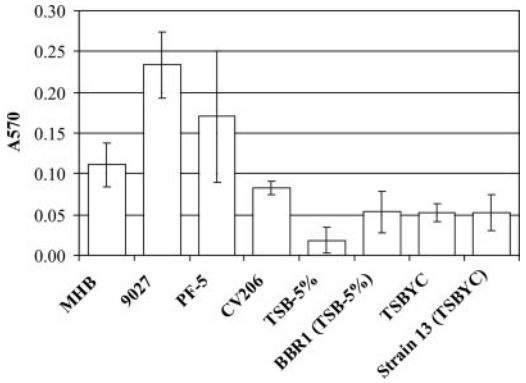

FIG. 6. CSFs from *Pseudomonas* spp. and *A. pyogenes* promote *C. jejuni* biofilm formation. Biofilm assays were performed with *C. jejuni* M129 in a growth medium consisting of 1:1 mixtures of MHB and CSF from *Pseudomonas aeruginosa* 9027, *Pseudomonas fluorescens* PF-5, *Chromobacterium violaceum* CV206, *A. pyogenes* BBR1, or *C. perfringens* strain 13. Trypticase soy broth (TSB) supplemented with 5% newborn calf serum (TSB-5%) and TSB supplemented with 0.5% yeast extract and 0.05% cysteine (TSBYC) were used as controls for *A. pyogenes* and *C. perfringens* CSFs, respectively. The source of the CSF or the type of control medium is shown on the *x* axis. Experiments were performed three times in triplicate, and error bars represent the standard deviation from the mean.

growth of *C. jejuni* (data not shown). In the presence of *Pseudomonas* and *Arcanobacterium pyogenes* BBR1 CSFs, an increase in biofilm development was observed, while CSFs from *Clostridium perfringens* and *Chromobacterium violaceum* had no apparent effect on biofilm development (Fig. 6).

Multiple *C. jejuni* isolates form biofilms. The ability of clinical and nonpathogenic *C. jejuni* isolates to form biofilms was determined. Biofilm formation did not appear to correlate to the pathogenicity of the *C. jejuni* isolate (Fig. 7). Isolate S2B was able to form biofilms to a degree similar to M129 and the other human clinical isolates. However, UMC3, a recent human clinical isolate, was the poorest biofilm former tested (Fig. 7). Interestingly, Joshua et al. recently reported that NCTC11168 did not attach to polystyrene (18). It was previously known that NCTC11168 loses motility on laboratory passage, and the disparate results may reflect such variation in the two isolates.

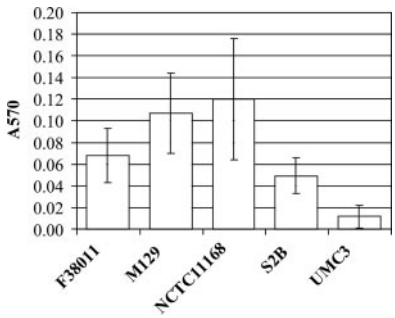

FIG. 7. Biofilm formation by *C. jejuni* isolates does not correlate with isolate virulence. Biofilm assays were performed with the *C. jejuni* type strain NCTC11168, human clinical isolates M129, F38011, and UMC3, and strain S2B. Experiments were performed three times in triplicate, and error bars represent the standard deviation from the mean.

DISCUSSION

The recognition of *Campylobacter jejuni* as an important human enteric pathogen has occurred only in the last 20 years, and to date, little is known about the pathogenesis and virulence factors of this organism. One of the least understood factors is the ability of *C. jejuni* to form biofilms on abiotic surfaces. The identification of environmental factors responsible for the initiation of *C. jejuni* biofilms is of primary importance in order to study the survival capabilities of this organism outside the host. Inhibition of environmental biofilm formation by this pathogen could potentially prevent the colonization of various animals domesticated for consumption and companionship and the contamination of our waterways that may also serve as sources of human infection.

In their natural environments, bacteria are often challenged by environmental stresses, including nutrient starvation, osmotic changes, temperature variation, and various oxygen tensions (13, 16, 17, 24, 25, 31, 32). Bacteria are thought to form biofilms when they sense environmental changes, which trigger the transition to a sessile lifestyle (17, 18, 24, 27, 30, 34). Other factors affecting biofilm formation include substratum properties, hydrodynamics, conditioning of the substratum, and characteristics of the bathing medium (3, 13, 24, 33). In some biofilm models, changes in ionic strengths and nutrient concentrations influenced the rate at which bacteria attached to and formed biofilms on a surface (13, 16, 17). Since environmental factors and the content of the medium can affect biofilm formation, we tested various bathing medium conditions for their effect on *C. jejuni* biofilms. More nutrient-rich media did not support optimal biofilm formation, suggesting that nutrient-poor environments, such as those found in watering systems, may promote *C. jejuni* biofilm formation. We also examined the responses of *C. jejuni* biofilm formation to various concentrations of the osmolytes, glucose, sucrose, and NaCl. Increasing levels of each of these osmolytes resulted in a significant decrease in *C. jejuni* biofilm formation. This decrease may be the result of the morphological transformation from rod- or spiral-shaped cells to those which are more coccoid. Coccoid forms may represent a degenerate cell form in which damage to the cell membrane and degradation of cellular components may take place during periods when osmoadaptation may be required (22).

Incubation temperature and oxygen tension can also influence biofilm formation. However, few studies have been conducted on the direct effect of aerobiosis on *C. jejuni* survival and biofilm formation. In marine environments, dissolved oxygen concentrations may be decreased by lower water flow rates, increased temperature, competing organic matter, and reduced turbulence (7). In keeping with the microaerophilic and thermophilic growth preferences of *C. jejuni*, lower oxygen tensions and higher temperatures increased biofilm formation, whereas high ambient temperatures under aerobic conditions inhibited biofilm formation. These results indicate that environmental conditions and the nutritional state of the medium can influence the ability of *C. jejuni* to form biofilms.

Physicochemical properties of the abiotic surface can also affect *C. jejuni* attachment. *C. jejuni* was able to attach to hydrophobic and hydrophilic surfaces to various degrees, with good biofilm formation being observed on hydrophobic surfaces, such as plastics found in watering systems. Bacterial cell surfaces are usually negatively charged, but hydrophobic surface components such as flagella and exopolysaccharides can help overcome the repulsive forces (13, 15, 17, 18, 30).

Other studies have shown that the treatment of bacteria with protein synthesis inhibitors can markedly decrease biofilm formation and cause the release of attached bacteria (3, 13, 26), suggesting the requirement for de novo protein synthesis. Preincubation of *C. jejuni* cells with Cm inhibited biofilm formation, suggesting that *C. jejuni* synthesizes proteins required for attachment and biofilm formation in response to appropriate signals and growth conditions.

The flagella of a number of bacterial species play a significant role in the rate of attachment to a surface and subsequent biofilm formation (13, 18, 26, 27). In this study, a *C. jejuni* flagellum-deficient mutant (M129::*flaAB*) showed reduced biofilm formation compared with the wild-type strain at later time points. These findings may suggest that *C. jejuni* flagella may be required for biofilm development and maturation rather than, or in addition to, attachment to a surface. The results are consistent with reports that aflagellate *C. jejuni* mutants are defective in pellicle formation and attachment to glass at the air-liquid interface (18).

Quorum sensing or cell-to-cell signaling has been documented to play a role in cell attachment to and detachment from a biofilm (13, 14, 36). *C. jejuni* M129 was grown in the presence of CSFs from other bacteria. The CSFs from *Pseudomonas* spp. and *A. pyogenes* increased *C. jejuni* biofilm formation. While *Pseudomonas* spp. produce homoserine lactone signals, *A. pyogenes* has a *luxS* homologue responsible for the production of AI-2 (S. J. Billington and B. H. Jost, unpublished data). However, the exact content of the CSFs is unknown, as is the component responsible for promoting biofilm formation. In *C. jejuni*, the production of AI-2 was recently described. This system is highly conserved in both gram-positive and gram-negative bacteria and is thought to be used for interspecies communication (14). The *luxS* gene encodes the final enzyme in the biosynthetic pathway for AI-2 production (14). To assess the role of the AI-2 quorum-sensing system in *C. jejuni* biofilm formation, a *C. jejuni luxS* mutant deficient in the production of AI-2 was constructed. Our studies suggest the possibility that *C. jejuni* biofilm development may be dependent on AI-2 since a reduction in biofilm formation was observed in the *luxS* mutant compared to that in the wild type. We observed an increase in the *luxS* mutant biofilm when grown in the presence of wild-type M129 CSF. While the exact nature of gene regulation during *C. jejuni* biofilm formation is not understood, clearly cell-to-cell communication via AI-2 plays a role in the induction of the expression of genes required for these functions. *Pseudomonas* spp., commonly found in the environment, and *A. pyogenes*, a common inhabitant of domestic and wild animals, may have a common signal that *C. jejuni* recognizes and uses to activate the gene transcription necessary for attachment and biofilm formation.

Biofilm formation in *C. jejuni* does not appear to correlate with the pathogenesis of the isolate. The ability of *C. jejuni* to form biofilms on an abiotic surface may help explain its ability to survive outside its normal host and act as a source of contamination for animals and humans. Consistent with this interpretation, *C. jejuni* aggregates, which may at least mimic bio-

film growth, confer increased survival compared to cells grown in the planktonic state (18).

Despite the environmental limitations of *C. jejuni* isolates, their survival in biofilms may play an important role in the transmission of the pathogen to animals and carcasses in husbandry and food processing plants, thus affecting humans. Biofilm variability among isolates could contribute to certain strains being of particular concern for human infections. Our studies should therefore be extended to determine the influence of watering distribution systems at these facilities and the correlation of *C. jejuni* isolates found in biofilms with those that colonize animals and cause human outbreaks.

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