microbial biotechnology

Microbial Biotechnology (2010) **3**(3), 242–258 doi:10.1111/j.1751-7915.2009.00118.x

Review article

Advances in *Campylobacter* biology and implications for biotechnological applications

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Summary

***Campylobacter jejuni* is a major foodborne pathogen of animal origin and a leading cause of bacterial gastroenteritis in humans. During the past decade, especially since the publication of the first *C. jejuni* genome sequence, major advances have been made in understanding the pathobiology and physiology of this organism. It is apparent that *C. jejuni* utilizes sophisticated mechanisms for effective colonization of the intestinal tracts in various animal species. Although *Campylobacter* is fragile in the environment and requires fastidious growth conditions, it exhibits great flexibility in the adaptation to various habitats including the gastrointestinal tract. This high adaptability is attributable to its genetically, metabolically and phenotypically diverse population structure and its ability to change in response to various challenges. Unlike other enteric pathogens, such as *Escherichia coli* and *Salmonella*, *Campylobacter* is unable to utilize exogenous glucose and mainly depends on the catabolism of amino acids as a carbon source. *Campylobacter* proves highly mutable in response to antibiotic treatments and possesses eukaryote-like dual protein glycosylation systems, which modify flagella and other surface proteins with specific sugar structures. In this review we will summarize the distinct biological traits of *Campylobacter* and discuss the potential biotechnological approaches that can be developed to control this enteric pathogen.**

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Introduction

Campylobacter is a microaerophilic Gram-negative bacterium and is among the leading causes of human gastroenteritis (Allos, 2001; Olson *et al*., 2008), responsible for 400–500 million cases of infection each year worldwide (Ruiz-Palacios, 2007). Campylobacteriosis in humans presents as diarrhoea, fever and abdominal cramps and is recognized as a major risk factor for the onset of Guillain-Barré syndrome, which is a serious post-infection complication characterized by acute and progressive neuromuscular paralysis (Allos, 2001). Among *Campylobacter* species, *Campylobacter jejuni* accounts for more than 92% of human infections (Friedman *et al*., 2000; Gillespie *et al*., 2008) with an infectious dose as low as 500 bacteria (Robinson, 1981). The organism is transmitted to humans through a variety of sources, such as contact with infected pets or consumption of contaminated water or milk. However, the most frequent source of *C. jejuni* infection is contaminated, undercooked poultry meat (Olson *et al*., 2008). *Campylobacter*-induced enteritis is usually self-limiting and does not require antimicrobial treatment, but clinical therapy, often using erythromycin or fluoroquinolone antibiotics, is warranted for cases involving high fever, bloody diarrhoea or immunocompromised patients (Blaser and Engberg, 2008). The increasing prevalence of drug-resistant *Campylobacter* has compromised the effectiveness of these antibiotics and poses a significant threat to public health in many countries (Blaser and Engberg, 2008).

Facilitated by the decoding of genome sequences, our understanding of *Campylobacter* as a zoonotic pathogen significantly advanced in the last decade. The progress is especially prominent in the areas of metabolic pathways, genetic variability, adaptation mechanisms, protein glycosylation systems, colonization factors and molecular basis of antibiotic resistance. These recent advances reveal distinct features of this pathogenic organism, provide new insights into *Campylobacter* physiology and pathobiology, and open potential avenues for the development of

intervention strategies to prevent and control *Campylobacter* colonization in animal reservoirs and infection of the human host. This review will summarize these advances with a particular emphasis on the unique aspects of *Campylobacter* biology. The implications of these findings for biotechnological applications will also be discussed. Detailed discussion on *Campylobacter* pathogenesis and host immune responses has been presented in several recent review papers (Young *et al.*, 2007; Janssen *et al.*, 2008; Poly and Guerry, 2008; Zilbauer *et al.*, 2008) and will not be a focus of this review.

Central metabolism and carbon flux surrounding pyruvate

With the exception of 6-phosphofructokinase (Pfk), *C. jejuni* maintains a complete Embden-Meyerhof-Parnas (EMP) pathway and citric acid cycle (CAC) (Parkhill *et al.*, 2000). Although *C. jejuni* possesses a fructose-1,6-bisphosphatase orthologue (Fbp) (Velayudhan and Kelly, 2002), the lack of Pfk results in the inability to catalyse the conversion of fructose-6-phosphate to fructose-1,6-bisphosphate (Fig. 1) and consequently renders this organism unable to utilize exogenous glucose (Vandamme *et al.*, 2005). Pyruvate is a central metabolite connecting the EMP pathway and CAC, but is not formed from glucose in *C. jejuni*; yet acetyl-CoA is still formed in the cell (Mendz *et al.*, 1997; St Maurice *et al.*, 2007). Many anaerobes produce acetyl-CoA from the oxidative decarboxylation of pyruvate catalysed by pyruvate : ferredoxin oxidoreductase (PFOR) and reduce ferredoxin (White, 2007). Different from these anaerobes, members of the ε-proteobacteria including *C. jejuni* use flavodoxin as the preferred electron carrier (Cremades *et al.*, 2005). *Campylobacter jejuni* homologues of flavoprotein (FldA; Cj1382c) and flavodoxin : quinole reductase (Fqr; Cj0559) are likely involved in this process.

An endogenous source of pyruvate is the catabolism of glucogenic amino acids producing α-keto or α-oxoacids (Fig. 1). Thus, amino acids likely play an important role in *Campylobacter* metabolism (Guccione *et al.*, 2008). Several amino acids, particularly L-aspartate, L-glutamate, L-serine and L-proline, are significantly depleted from the culture media by *C. jejuni* strain NCTC 11168 (Guccione *et al.*, 2008), and the ability to utilize L-asparagine and glutathione is highly strain-specific

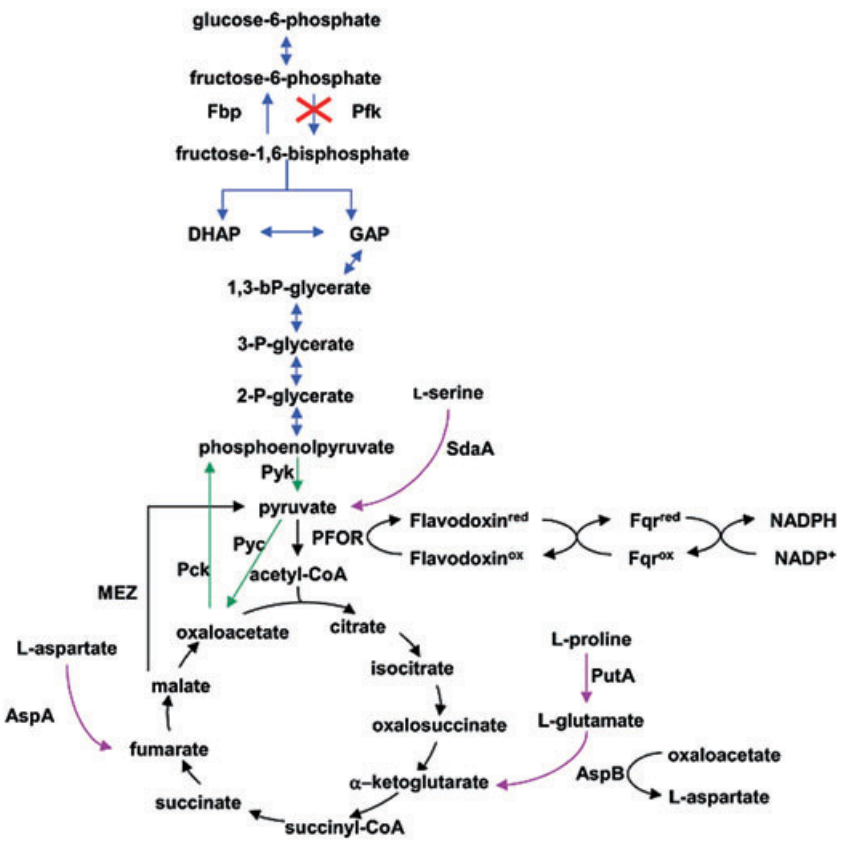

Fig. 1. Central metabolism of *C. jejuni* and carbon flux surrounding pyruvate. Pathways and reactions are distinguished by the colour of arrows connecting intermediates; blue, possible anabolic role of EMP pathway; magenta, preferred amino acid catabolism; green, potential futile cycle; black, oxidative citric acid cycle and pyruvate carboxylation/decarboxylation. This composite figure is adapted from previously published information (Velayudhan and Kelly, 2002; St Maurice *et al.*, 2007; Guccione *et al.*, 2008).

(Hofreuter *et al*., 2008). Transamination of alanine, possibly by putative aminotransferases (Cj0762c or Cj0150c), and dehydration of serine by SdaA (Velayudhan *et al*., 2004) will each produce pyruvate. Proline is converted to glutamate through a glutamate-5-semialdehyde intermediate (Cj1503c, PutA), and subsequent transamination of glutamate and oxaloacetate by AspB will produce α-ketoglutarate and aspartate (Guccione *et al*., 2008). The deamination of aspartate by AspA yields fumarate (Guccione *et al*., 2008). Fumarate and α-ketoglutarate, formed from these amino acids, can enter the CAC. Malate is converted to pyruvate by the malic enzyme (MEZ), and oxaloacetate is first converted to phosphoenolpyruvate (PEP) by phosphoenolpyruvate carboxykinase (PckA) and then to pyruvate by pyruvate kinase (Pyk) (Velayudhan and Kelly, 2002; Sauer and Eikmanns, 2005). The lack of a PEP synthase in *C. jejuni* suggests that decarboxylation of oxaloacetate by PckA is the main mechanism of PEP production (Velayudhan and Kelly, 2002). Consistent with this notion, a *pckA* mutant could not be constructed, thus *pckA* is likely an essential gene in *C. jejuni* (Velayudhan and Kelly, 2002). Although the lack of Pfk results in the inability to utilize exogenous glucose, the presence of an Fbp orthologue (Cj0840c) strongly suggests an anabolic role for the EMP pathway, requiring the synthesis of PEP. Anabolically formed glucose may have several metabolic fates, including incorporation into lipooligosaccharides (LOS), capsular polysaccharides (CPS), or protein glycans or entry into the pentose phosphate pathway. Interestingly, the *C. jejuni* genome encodes a functional pyruvate kinase (Pyk), posing a potential futile cycle between PEP, pyruvate and oxaloacetate and resulting in the net loss of one ATP (Velayudhan and Kelly, 2002). The role of Pyk in *Campylobacter* metabolism remains unclear, but may be involved in the catabolism of PEP derived from an unknown substrate that enters the EMP pathway subsequent to the formation of fructose-1,6-bisphosphate (Velayudhan and Kelly, 2002).

Aerobic and anaerobic respiration

The highly branched electron transport system of *C. jejuni* is capable of both anaerobic and aerobic respiration (Fig. 2), affording this organism the flexibility to respire under varying levels of available electron donors and terminal acceptors. Additionally, branches that use terminal oxidases with high oxygen affinity remove trace oxygen, thereby creating a low intracellular oxygen state that is conducive to the activity of oxygen sensitive enzymes and the use of alternative terminal electron acceptors. Early reports on the respiratory system of *C. jejuni* reported high activities when membrane vesicles were incubated with hydrogen and formate, moderate rates with malate, succinate and lactate, and poor activity with NADH as electron donors (Hoffman and Goodman, 1982). Sulfite, which is normally an inhibitory substance added to food as a preservative, is a respiratory substrate for *C. jejuni* (Myers and Kelly, 2005). A gluconate dehydrogenase (GADH) was recently reported to enable *C. jejuni* to respire using gluconate as an electron donor at elevated temperatures. Mutation of GADH led to a marked impairment in the colonization of poultry intestines (Pajaniappan *et al*., 2008). *Campylobacter jejuni* possesses a cluster of genes (*nuoA-N*) encoding homologues of the NADH : ubiquinone oxidoreductase complex (also known as NADH dehydrogenase or Complex I in other bacteria) (Parkhill *et al*., 2000). Most of the genes that encode

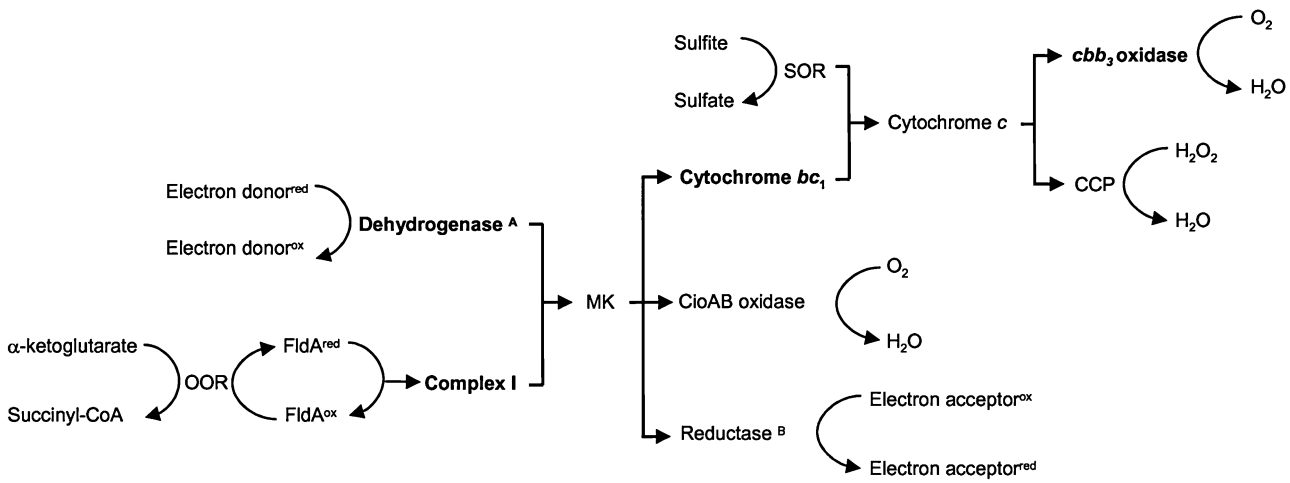

Fig. 2. Schematic diagram of the highly branched respiratory pathway in *C. jejuni*. [A]Primary dehydrogenases that oxidize formate, hydrogen, lactate and succinate. Electrons from gluconate likely enter at the level of cytochrome *c*. [B]Reductases that transfer electrons onto fumarate, nitrate, nitrite and S-and N-oxides. Bold type: potential coupling sites associated with proton translocation and energy conservation. MK, Menaquinone pool.

Complex I are intact except for homologues of the NADH dehydrogenase subunits (*nuoE* and *nuoF*), accounting for the poor respiratory ability of *C. jejuni* using NADH as an electron donor (Hoffman and Goodman, 1982). In their place within the operon are two genes (*Cj1574c* and *Cj1575c*) of unknown function (Weerakoon and Olson, 2008), and it is hypothesized that Cj1574c and Cj1575c accept electrons from reduced flavoprotein (FldA), rather than NADH (Weerakoon and Olson, 2008). Analysis of the *C. jejuni* NCTC 11168 genome revealed no α-ketoglutarate dehydrogenase homologue; however, OOR (encoded by *oorDABC*) is a functionally equivalent redox complex that catalyses the conversion of α-ketoglutarate to succinyl-CoA (Hughes *et al*., 1998) and transfers electrons to FldA (Weerakoon and Olson, 2008), providing a source of electrons for Complex I.

In *C. jejuni*, the electron flow from donor to acceptor, through dehydrogenases to reductases, and oxidases, is carried by lipid-soluble menaquinone and two types of cytochromes (*b*- and *c*-type). Although menaquinone can be detected from members of the *Campylobacter* genus (Moss *et al*., 1984), the *C. jejuni* genome reveals no obvious homologues to the *Escherichia coli* MenZ-G enzymes catalysing the conversion of chorismate to menaquinone (Parkhill *et al*., 2000). An alternative pathway for menaquinone synthesis, termed the futalosine pathway, was recently described in *Streptomyces coelicolor* (Hiratsuka *et al*., 2008). This study suggested a role for this alternative pathway in menaquinone synthesis in *C. jejuni*.

Campylobacter is able to respire both aerobically and anaerobically, utilizing oxygen or alternative electron acceptors to terminate the electron transport chain. *Campylobacter jejuni* possesses two terminal oxidases, including a high-affinity cbb_3-type cytochrome *c* oxidase and a cyanide insensitive oxidase (Smith *et al*., 2000). In terms of aerobic respiration through the high-affinity oxidase, electrons fed into the menaquinone pool are transferred to cytochrome bc_1 (Complex III) then to a periplasmic cytochrome *c* (Fig. 2). The cbb_3-type oxidase (CcoNOPQ; Cj1487c-Cj1490c) has very high affinity for oxygen (K_m = 40 nM) (Jackson *et al*., 2007), which allows *C. jejuni* to aerobically respire under oxygen-limiting conditions such as those encountered in a host's intestine. The second branch at the terminal oxidase level of aerobic respiration is mediated by CioAB (cyanide insensitive oxidase, formerly CydAB) and has a low affinity to oxygen (Jackson *et al*., 2007). The CioA mutants colonized the poultry intestine as efficiently as the wild-type, but CcoN mutants were completely impaired in colonization indicating a significant role for oxygen during *in vivo* colonization (Weingarten *et al*., 2008). Although the conditions encountered by *C. jejuni* within the animal host may be considered anaerobic, it is plausible that oxygen perfusion from actively respiring host epithelial cells provide an oxygen gradient in the mucosal layer approaching anoxic conditions near the intestinal lumen; thus, the role of terminal oxidases, especially the high-affinity cbb_3-type, in the animal host may be energy conservation through scavenging host-provided oxygen (Weingarten *et al*., 2008).

In addition to aerobic respiration, *C. jejuni* is capable of anaerobic respiration. The electron transfer from cytochrome *c* to H_2O_2 is facilitated by two different periplasmic cytochrome *c* peroxidases (CCP; Cj0020c and Cj0358). Although Cj0358 plays only a minor role in colonization (Bingham-Ramos and Hendrixson, 2008), Cj0020c plays an important role in chicken colonization and was named *docA* (determinant of colonization) (Hendrixson and Dirita, 2004). The contribution of periplasmic CCP to colonization remains unclear, but may be involved in the removal of endogenously produced H_2O_2 during formate respiration (Atack and Kelly, 2007). Anaerobic respiration in *C. jejuni* is also accomplished by a variety of reductases that transfer electrons from the menaquinone pool onto fumarate (FrdABC; Cj0408-Cj0410), nitrate (NapAB; Cj0780 and Cj0781), nitrite (NrfA; Cj1357c) and N- and S-oxides (TorAC; Cj0264c and Cj0265c) (Sellars *et al*., 2002; Pittman *et al*., 2007). While the high-affinity cbb_3-type terminal oxidase plays a significant role in the colonization of poultry intestines, the role of terminal reductases is less clear. TorA plays a minimal role in poultry colonization (Weingarten *et al*., 2008) with conflicting evidence presented for respiration using nitrate (Pittman *et al*., 2007; Weingarten *et al*., 2008). These discrepancies may be attributed to genetic differences among strains, inoculum dose, or diet fed to the chickens and microbiota of the chicken intestine affecting the production or availability of terminal electron acceptors. The versatile respiratory system of *Campylobacter* provides for the cell's energy demands and detoxifies potentially damaging compounds, such as nitrite, nitric oxide and sulfite.

Dual protein glycosylation systems in *C. jejuni*

Campylobacter jejuni possesses both *O*-linked and *N*-linked protein glycosylation systems (Szymanski *et al*., 2005; Guerry and Szymanski, 2008). The *N*-linked protein glycosylation occurs at the carboxamide side-chain of asparagine, while the *O*-linked protein glycosylation system mediates the attachment of glycans to the hydroxyl group of serine or threonine residues on a protein. Flagellin is the only *O*-glycoprotein in *C. jejuni* and is extensively modified with pseudaminic acid (Thibault *et al*., 2001). In *C. jejuni* NCTC 11168, the DNA locus responsible for *O*-linked protein glycosylation consisting of about 50 genes, and the glycosylation locus is highly

variable among different strains of *C. jejuni* (Karlyshev *et al.*, 2005). Mutations eliminating glycans from flagella reduced *Campylobacter* adherence to and invasion of INT407 cells, decreased auto-agglutination and attenuated diarrhoea in a ferret animal model (Guerry *et al.*, 2006). Thus, the *Campylobacter* flagella require glycosylation to carry out its virulence-associated functions (Guerry *et al.*, 2006). It was believed that *N*-linked glycoproteins were exclusive to eukaryotes; however, recent evidence of this post-translational modification has been documented in archaea and eubacteria (Schaffer *et al.*, 2001). The first bacterial *N*-linked glycosylation pathway was reported in *C. jejuni* (Szymanski *et al.*, 1999), and *Campylobacter* is the only bacterium known to possess both *O*- and *N*-linked protein glycosylation systems to date (Szymanski *et al.*, 2005). *N*-linked protein glycosylation in *C. jejuni* is carried out by the *pgl* locus consisting of 11 genes (*pglA-F*, *pglH-K* and *gne*) (Szymanski *et al.*, 1999; Linton *et al.*, 2002; Alaimo *et al.*, 2006; Kelly *et al.*, 2006). Analogous to dolichyl-phosphate in eukaryotes, undecaprenyl-phosphate serves as a carrier for the cytosolic addition of UDP-activated oligosaccharides (Feldman *et al.*, 2005). PglD, PglE and PglF convert UDP-*N*-acetyl glucosamine (GlcNAc) to UDP-bacillosamine that is attached to the undecaprenyl pyrophosphate by PglC. The *gne* gene encodes an epimerase that interconverts glucose and GlcNAc to galactose and *N*-acetyl galactosamine (GalNAc), respectively. Sequential addition of GalNAc residues are achieved by the transferase activities of PglA, PglJ and PglH, with PglI forming the branching β1,3 bond to glucose (Glover *et al.*, 2005; Linton *et al.*, 2005). The entire heptasaccharide is flipped across the cytoplasmic membrane into the periplasm by PglK (formerly WlaB) (Alaimo *et al.*, 2006; Kelly *et al.*, 2006), and transferred to the Asn residue of the D/E-X_1-N-X_2-S/T (X_1 and X_2 are any amino acids other than proline) glycan accepting sequon by PglB, an oligosaccharyl transferase (Kowarik *et al.*, 2006). The bacterial glycan acceptor sequon requires the negatively charged side-chain of aspartate or glutamate at the −2 position, which is not required by eukaryotes, suggesting that *C. jejuni* has a more specific attachment site of the *N*-glycan than eukaryotes (Kowarik *et al.*, 2006). Interestingly, the *C. jejuni pgl* gene cluster also functionally mediates *N*-linked protein glycosylation in *E. coli* and can modify proteins with diverse glycan structures (Feldman *et al.*, 2005; Wacker *et al.*, 2006).

Genetic and phenotypic instability

A distinct feature of *Campylobacter* is its genetic and phenotypic instability resulting from high-frequency mutation and horizontal gene transfer. The instability results in remarkable strain-to-strain variations, creating a significant obstacle for deciphering gene functions in *Campylobacter*. The high mutation rate is attributable to an incomplete mismatch DNA repair system. The *mutH*, *mutL*, *mutS* and *uvrD* genes are key components of the mismatch repair system in bacteria, and mutants defective in any of these genes show a hypermutable phenotype with a 100- to 1000-fold increase in mutation frequency (Miller, 1996). *Campylobacter jejuni* does not have *mutH, mutL* and *uvrD* homologues, but has a variant copy of *mutS* (*mutS2*) (Parkhill *et al.*, 2000). MutS2, however, is not involved in mismatch repair and was shown to suppress intergenomic recombination in *Helicobacter pylori* (Kang *et al.*, 2005). The inability of *C. jejuni* to recognize and repair mismatch errors through the MutHLS complex may lead to the observed high frequency of spontaneous point mutations and phase variation, contributing to genetic diversity in *Campylobacter*.

Horizontal gene transfer is also involved in generating genetic variation in *Campylobacter*. Comparative genomic studies revealed that *C. jejuni* has hypervariable gene clusters, which are located on chromosome and functionally associated with biosynthesis of surface structures (LOS, CPS and flagella) and restriction–modification systems (Parkhill *et al.*, 2000; Taboada *et al.*, 2004). These hypervariable clusters have a different G+C composition from that of the rest of the chromosome (Parkhill *et al.*, 2000; Hofreuter *et al.*, 2006), suggesting that horizontal gene transfer may be responsible for their acquisition. Natural transformation, conjugation and transduction are the mechanisms of horizontal gene transfer in bacteria, and *C. jejuni* can use all three mechanisms. Natural transformation significantly contributes to genetic diversity and the spread of antibiotic resistance determinants in this bacterium. For example, co-culture or co-colonization of *C. jejuni* strains harbouring different antibiotic resistance markers generated *C. jejuni* populations possessing resistance to multiple antibiotics, and this process was prevented in the Cj1211 mutant that was deficient in natural transformation (de Boer *et al.*, 2002; Jeon *et al.*, 2008). Although a complete understanding of the overall mechanism of natural transformation is still lacking and the recognition sequence for natural transformation has not been identified in *Campylobacter*, multiple factors contributing to this process have been reported (Guerry *et al.*, 1994; Wiesner *et al.*, 2003; Larsen *et al.*, 2004; Jeon and Zhang, 2007; Jeon *et al.*, 2008; 2009).

Compared with natural transformation, limited information is available for conjugation and transduction in *Campylobacter*. *Campylobacter jejuni* strain RM1221 contains a Mu-like prophage located in its chromosome (Fouts *et al.*, 2005), which was not found in the genome sequence of *C. jejuni* NCTC 11168 (Parkhill *et al.*, 2000). The Mu-like bacteriophage present in strain RM1221 appears to be widely prevalent in many *C. jejuni* and *C.*

coli strains and contributes to the genetic diversity of this genus (Parker *et al*., 2006; Clark and Ng, 2008). Recently, evidence of intragenomic and interstrain recombination, mediated by bacteriophages, was reported in *C. jejuni* (Scott *et al*., 2007a,b). Conjugative plasmids have been described in *Campylobacter* (Taylor *et al*., 1981; Bacon *et al*., 2000), which were transferrable between different strains in culture media and in the avian intestine (Avrain *et al*., 2004). The exact contribution of conjugation and transduction to genetic diversity in *Campylobacter* awaits further investigation.

Flagellum-mediated motility and secretion of virulence factors

The motility of *Campylobacter* is imparted by polar flagella and is recognized as an important virulence factor. Challenge of human volunteers with a mixture of motile and non-motile *C. jejuni* resulted in the isolation of only motile *C. jejuni* (Black *et al*., 1988), implying a significant role of motility in colonization. Flagella are involved in auto-agglutination (Misawa and Blaser, 2000) and biofilm formation (Joshua *et al*., 2006; Kalmokoff *et al*., 2006). Structurally, the flagellar filament consists of flagellin subunits encoded by two tandem genes, *flaA* and *flaB* (Nuijten *et al*., 1990; Guerry *et al*., 1991). While the expression of *flaA*, encoding the major flagellin (FlaA) subunit, is σ^{28}-dependent (Nuijten *et al*., 1990; Guerry *et al*., 1991), *flaB* is expressed by a σ^{54}-dependent promoter and produces the minor flagellin (FlaB) subunit (Guerry *et al*., 1991). FlaA and FlaB are highly immunogenic (Shoaf-Sweeney *et al*., 2008), although immune recognition is independent of toll-like receptor 5 (Andersen-Nissen *et al*., 2005). Mutation of *flaA*, but not *flaB*, reduced motility significantly, rendered *Campylobacter* less invasive to human INT407 cells and decreased the level of chicken colonization (Guerry *et al*., 1991; Wassenaar *et al*., 1991; 1993). Although the *flaA* mutation affected both auto-agglutination and biofilm formation, *flaB* is only involved in biofilm formation but not auto-agglutination (Golden and Acheson, 2002; Kalmokoff *et al*., 2006). Analysis of the genome sequence of *C. jejuni* identified two additional flagellin paralogues, *flaC* (*Cj0720c*) and *flaD* (*Cj0887c*) (Parkhill *et al*., 2000). Inactivation of *flaC* did not affect motility, but reduced invasion of HEp-2 cells (Song *et al*., 2004) and pellicle formation (Kalmokoff *et al*., 2006). Similar to the *flaA* mutation, the *flaD* mutation caused defects in motility and auto-agglutination; however, FlaD is not detected in the flagellar filament of *C. jejuni* (Golden and Acheson, 2002).

Flagellar expression is subject to phase variation and contributes to the unstable motile phenotype in *Campylobacter.* Phase variation between motile and non-motile phenotypes occurs frequently *in vitro* and *in vivo* (Caldwell *et al*., 1985; Karlyshev *et al*., 2002; Hendrixson, 2006; 2008), and is mediated by slipped-strand mispairing primarily in homopolymeric or occasionally heteropolymeric tracts, resulting in frameshift mutations in the genes associated with flagellar biosynthesis and regulation (*flhA*, *flgS*, *flgR* and *maf*) (Park *et al*., 2000; Karlyshev *et al*., 2002; Hendrixson, 2006; 2008). The high-frequency phase variation was attributed to the A/T-rich genetic content of the *C. jejuni* chromosome and the absence of an intact mismatch repair system (Hendrixson, 2006).

The flagellar apparatus in *C. jejuni* is involved in secretion of virulence-associated proteins, such as Cia, FlaC and FspA. Cia (*Campylobacter* invasion antigen) proteins are associated with *C. jejuni* invasion of INT407 cells (Konkel *et al*., 1999), and their secretion requires the function of the flagellar basal body, hook and filament (Konkel *et al*., 2004). Mutation of the basal rod (*flgF*) and hook (*flgE*) elements prevented the secretion of FlaC, suggesting that FlaC is secreted via the flagellar apparatus. Secreted FlaC binds to HEp-2 cells and the FlaC mutant demonstrated reduced invasion compared with the wild-type strain (Song *et al*., 2004). FspA (flagellar secreted protein), a small acidic protein, is secreted by the flagellar system in *C. jejuni* (Poly *et al*., 2007). Although the exact function of FspA is unknown, a variant of FspA (FspA2) induced apoptosis in INT407 cells. Given the fact that *C. jejuni* lacks a typical type III secretion system, the flagellar secretion apparatus is likely an alternative mechanism for secretion of virulence factors in this pathogen.

Adaption to environmental stresses

As a fastidious, asaccharolytic and microaerobic bacterium, *C. jejuni* utilizes elaborate adaptation mechanisms to survive environmental stresses (Murphy *et al*., 2006), such as temperature shift, oxygen tension and nutrient depletion, which occur during transmission between the environment and animal hosts and within the host's intestine. The minimal temperature at which *C. jejuni* can grow is between 31°C and 32°C, with an optimal growth temperature of 42°C (Hazeleger *et al*., 1998). Unlike *E. coli*, *C. jejuni* lacks the genes encoding the key components of cold shock response such as CspA (Parkhill *et al*., 2000), which facilitates protein synthesis by blocking the formation of unfavorable secondary structures in mRNAs during cold shock (Jiang *et al*., 1997). Temperature downshift to below the minimal growth temperature resulted in massive downregulation of gene expression, including those involved in biosynthesis of macromolecules; however, some genes encoding heat shock proteins, chaperones and energy metabolism were upregulated, suggesting that *C. jejuni* utilizes active mechanisms for adaptation to low temperatures (Moen *et al*., 2005). Tem-

perature upshift (from 37°C to 42°C) led to upregulation of genes encoding heat shock proteins, chaperones and chaperonins, and downregulation of genes encoding ribosomal proteins (Stintzi, 2003). These findings suggest that with both temperature upshift and downshift *C. jejuni* reduces energy consumption and invokes the heat shock protection mechanism.

RpoS, a sigma factor associated with stationary phase response, is absent in *C. jejuni* (Parkhill *et al*., 2000). Despite this fact, active transcriptomic, metabolic and phenotypic changes occur in *Campylobacter* during the transition from exponential to stationary phase, including downregulation of genes associated with electron transport and protein synthesis, induction of protection mechanisms (heat shock response and oxidative stress resistance), shift in nutrient utilization and increase in motility (Wright *et al*., 2009). For effective colonization and infection, *C. jejuni* must be able to survive acid stresses in the stomach. Exposure to acid stress *in vitro* and *in vivo* (piglet stomach) induced the expression of genes involved in heat shock response and oxidative stress resistance (Reid *et al*., 2008). DNA microarray profiles of *Campylobacter* transcripts recovered from rabbit ileal loops, into which *C. jejuni* was directly inoculated without passing through the stomach, showed marked upregulation of the genes involved in oxidative stress response, heat shock response, iron metabolism, bile resistance (*cmeABC*), flagellar biosynthesis and the stringent response (Stintzi *et al*., 2005), suggesting that *C. jejuni* utilizes sophisticated mechanisms for adaptation in the intestinal environment. SpoT, a key regulator for stringent response, modulates *Campylobacter* survival in stationary phase and the resistance to oxidative stress and rifampicin (Gaynor *et al*., 2005). Expression of *spoT* was upregulated upon contact with INT407 cells and inactivation of *spoT* in *Campylobacter* reduced adherence, invasion and intracellular survival (Gaynor *et al*., 2005). Together, these findings suggest that modulating the expression of genes involved in heat shock response and oxidative stress may be a common mechanism utilized by *Campylobacter* to adapt to various stresses.

Mechanisms for *in vivo* colonization

As a zoonotic pathogen, *Campylobacter* colonizes the gastrointestinal tracts of a variety of animal hosts, either as a commensal or as a pathogen. Understanding the mechanisms *Campylobacter* uses for colonization may yield control strategies. In particular, *C. jejuni* colonization of the chicken intestinal tract has received considerable attention because faecal contamination of chicken carcasses during the slaughter process poses a significant risk for human exposure. To date, flagella and motility are the most well-defined colonization factors, and it is clear that non-motile *Campylobacter* cannot establish colonization in animal intestinal tracts. In addition, recent work also identified other factors that are required for effective colonization.

Adherence to epithelial cells of the intestine may be required for *C. jejuni* to resist intestinal peristalsis and expulsion. Several proteins have been identified in *Campylobacter* that mediate adherence to cultured cells including flagella (McSweegan and Walker, 1986), CadF (*Campylobacter* adhesion to fibronectin) (Konkel *et al*., 1997), PEB1a (Pei *et al*., 1998), PEB4 (Asakura *et al*., 2007) and JlpA (jejuni lipoprotein A) (Jin *et al*., 2001). Proteomic analysis demonstrated that FlaA, CadF, PEB1a and PEB4 are associated with the membrane fraction and are recognized by convalescent human sera (Cordwell *et al*., 2008). Consistent with their function in cellular adherence, most of these proteins are associated with the colonization of animal hosts. For example, mutations of *peb1a* and *peb4* decreased murine colonization (Pei *et al*., 1998; Asakura *et al*., 2007), and a CadF mutant demonstrated impaired colonization in chickens (Ziprin *et al*., 1999).

Bile is normally present in intestinal tracts and is a natural detergent with antimicrobial activities. Thus, resistance to bile is an essential mechanism for enteric bacteria to colonize animal intestines. In *Campylobacter*, bile resistance is primarily mediated by the CmeABC multidrug efflux pump (Lin *et al*., 2002; 2003). The CmeABC mutant was completely impaired for colonization of the chicken intestine due to the drastically increased sensitivity to bile compounds (Lin *et al*., 2003). Consistent with its important role in bile resistance and *in vivo* colonization, *cmeABC* is inducible by bile salts in culture media (Lin *et al*., 2005b). The induced expression of *cmeABC* in the intestine was also demonstrated in a rabbit ileal loop model using DNA microarray (Stintzi *et al*., 2005). These findings indicate that CmeABC is an important factor for *Campylobacter* adaptation in bile-containing environments including the intestinal tract.

Another important determinant for successful colonization is the ability to acquire adequate nutrients *in vivo*. Amino acids are the primary carbon source for *Campylobacter* with the preferred use of aspartate, glutamate, serine and proline (Guccione *et al*., 2008). Mutation of genes involved in the catabolism of aspartate (*aspA*) (Guccione *et al*., 2008) and serine (*sdaA*) (Velayudhan *et al*., 2004) reduced chicken colonization. Additionally, *sdaA* expression is upregulated during chicken colonization (Woodall *et al*., 2005), further suggesting an important role for serine catabolism during intestinal colonization. Recently, strain-specific utilization of asparagine, glutamine and glutathione was described in *C. jejuni* strain 81-176, and the ability to utilize these substrates was correlated with enhanced colonization of specific

tissues in mice (Hofreuter *et al.*, 2008). The strain-dependent substrate utilization is due to the presence of γ-glutamyl transpeptidase (GGT) and an exported form of asparaginase (AnsB) in *C. jejuni* 81-176. The *ggt* gene is also present in *C. jejuni* strain 81116 and mutation of this gene significantly reduced the duration of *C. jejuni* colonization in murine and avian hosts (Hofreuter *et al.*, 2006; Barnes *et al.*, 2007). In addition, signature-tagged transposon mutagenesis identified multiple genes contributing to *C. jejuni* colonization in chickens and some of the identified genes encode amino acid transporters (Hendrixson and Dirita, 2004).

The *C. jejuni* glycome also contributes to colonization of animal intestines. Mutation of genes necessary for *N*-linked protein glycosylation reduced *Campylobacter* colonization in chickens (Hendrixson and Dirita, 2004; Jones *et al.*, 2004; Karlyshev *et al.*, 2004; Kelly *et al.*, 2006). Mutagenesis of all putative *N*-glycosylated proteins in *C. jejuni* identified Cj1496c as a colonization determinant, but glycosylation of this protein did not seem to be required for its function in colonization (Kakuda and Dirita, 2006). Capsular polysaccharides influence *Campylobacter* colonization and virulence. A *kpsM* mutant of *C. jejuni* strain NCTC 11168 demonstrated a complete lack of colonization in chickens (Jones *et al.*, 2004), and a *kpsM* mutation in a different strain (81-176) reduced the invasion of INT 407 cells and decreased the development of diarrhoea in the ferret model (Bacon *et al.*, 2001).

Multiple two-component regulatory systems (12 response regulators and 7 sensor kinases in strain NCTC 11168) have been identified in *C. jejuni* (Parkhill *et al.*, 2000; Fouts *et al.*, 2005), and several of them were shown to play a role in intestinal colonization. RacRS (reduced ability to colonize) modulates *Campylobacter* gene expression in a temperature-dependent manner. The *racR* mutation reduced bacterial growth at 42°C and impaired *C. jejuni* colonization in chickens (Bras *et al.*, 1999). Inactivation of DccRS (diminished capacity to colonize) did not impair the growth of *Campylobacter* in culture media, but resulted in a deficiency in the colonization of mice and chickens (MacKichan *et al.*, 2004). Inactivation of CbrR (*Campylobacter* bile resistance regulator) rendered *Campylobacter* more sensitive to bile salts than the wild-type strain and significantly reduced chicken colonization, presumably due to the increased sensitivity to bile (Raphael *et al.*, 2005). The cognate sensor kinase for CbrR and how it regulates bile resistance are still unknown. FlgRS is a two-component regulatory system associated with the expression of flagellin subunit genes (Hendrixson and Dirita, 2003; Wösten *et al.*, 2004), and mutation of FlgRS reduced the colonization level in chickens (Hendrixson and Dirita, 2004; Wösten *et al.*, 2004). These findings suggest that two-component regulatory systems are important for *Campylobacter* adaptation in the intestinal tract, but in most cases the exact environmental stimuli recognized by the two-component systems are unknown.

In addition to two-component regulatory systems, *C. jejuni* also utilizes other transcriptional regulators for adaptation and colonization. Some examples include Fur and CmeR. As a transcriptional repressor, Fur controls iron homeostasis and modulates the expression of multiple genes in *C. jejuni* (van Vliet *et al.*, 1998; Palyada *et al.*, 2004). Mutation of Fur significantly reduced the colonization of *Campylobacter* in chickens (Palyada *et al.*, 2004). CmeR belongs to the TetR family of transcriptional regulators (Ramos *et al.*, 2005). In *Campylobacter,* CmeR functions as a repressor for CmeABC and modulates the expression level of this efflux pump (Lin *et al.*, 2005a). A recent study using DNA microarray also revealed that CmeR is a pleiotropic regulator affecting the expression of multiple genes and is required for optimal colonization of *Campylobacter* in chickens (Guo *et al.*, 2008). The regulation of *cmeABC* by CmeR is through direct binding of CmeR to the promoter region of *cmeABC* (Lin *et al.*, 2005a). Bile compounds inhibit the binding of CmeR to the promoter DNA of *cmeABC* (Lin *et al.*, 2005b), which explains why bile salts induce the expression of *cmeABC*. The recent crystallization work revealed a two-domain structure of CmeR, including a DNA-binding motif formed by the N-termini of the CmeR dimer and a large tunnel-like ligand-binding cavity in the C-terminal domain of each monomer (Gu *et al.*, 2007; Routh *et al.*, 2009). The tunnel is surrounded by mostly hydrophobic residues and is predicted to be able to accommodate large, negatively charged bile acid molecules. Based on the structural data and the fact that bile compounds induced the expression of CmeR-regulated genes (Guo *et al.*, 2008), it is speculated that bile acids and potentially other unidentified signals in the gut interact with the ligand-binding pocket of CmeR, which triggers a conformational change in the DNA-binding domain, leading to the release of CmeR from its target promoters. A model illustrating the regulatory mechanism of CmeR is shown in Fig. 3.

Mechanisms of antibiotic resistance

Campylobacter has developed resistance to multiple antibiotics and the general resistance mechanisms to clinically important antibiotics are illustrated in Fig. 4 and reviewed elsewhere (Zhang and Plummer, 2008). Depending on their chemical and structural properties, antibiotics enter bacterial cells through different routes. Small hydrophilic antibiotics can pass through membrane porins, while hydrophobic antibiotics diffuse through the lipid membrane (Nikaido, 2003). The first obstacle for antibiotics is cell permeability, which is affected by various membrane and surface structures. *Campylobacter jejuni* has both LOS and CPS, which contribute to the hydrophilic nature of the

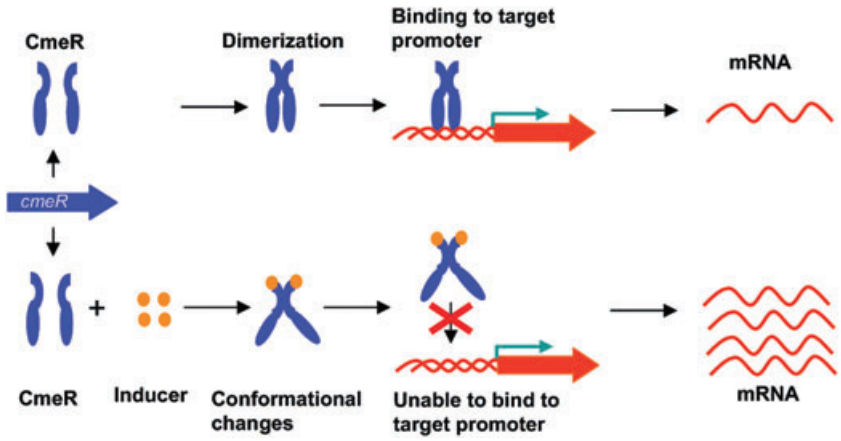

Fig. 3. CmeR regulation of gene expression in *Campylobacter*. In the absence of an inducer, dimeric CmeR binds to target promoters and inhibit the expression of target genes (e.g. *cmeABC*). In the presence of an inducer (e.g. bile salts), the interaction of an inducer with the C-terminal ligand-binding pocket of CmeR triggers a conformational change in the N-terminal DNA binding domain, preventing the binding of CmeR to target promoters and leading to enhanced expression of target genes.

bacterial surface. A recent study demonstrated that mutagenesis of LOS, but not CPS, significantly sensitized *C. jejuni* (strain NCTC 11168) to erythromycin, a hydrophobic macrolide antibiotic, suggesting that LOS reduces the permeability to hydrophobic antibiotics (Jeon *et al.*, 2009). Once antimicrobials traverse the cytoplasmic membrane, they are extruded by multidrug efflux transporters, which reduce the intracellular drug concentration and constitute a second defence mechanism against antibiotics. Although the genome sequences of *C. jejuni* revealed the presence of multiple putative drug efflux transporters of different families, most of them have not been functionally characterized. CmeABC, an RND-type efflux transporter, appears to be the primary drug efflux pump in *Campylobacter* and confers resistance to structurally diverse antibiotics as well as bile compounds (Lin *et al.*, 2002; Pumbwe and Piddock, 2002). As discussed earlier, CmeABC is also required for *Campylobacter* colonization in the chicken intestine

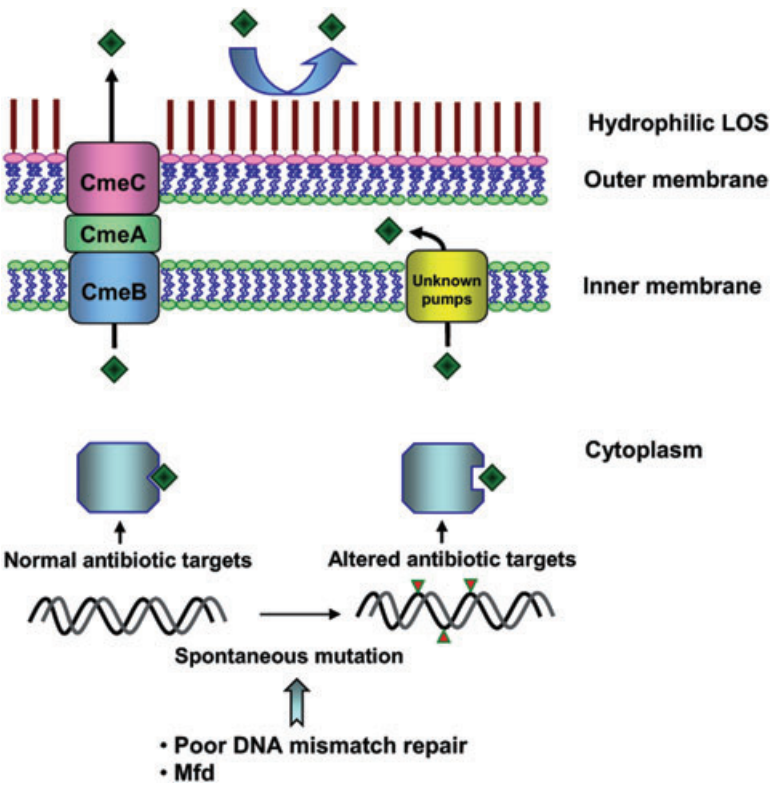

Fig. 4. Mechanisms associated with *Campylobacter* resistance to macrolide and fluoroquinolone antibiotics. LOS reduces the uptake of hydrophobic antibiotics (e.g. macrolide); efflux pumps (such as CmeABC and other uncharacterized efflux transporters) decrease the intracellular concentration of antibiotics; and chromosomal mutations reduce the affinity of antibiotics to their targets. Mfd and the lack of an intact mismatch repair system enhance the spontaneous mutation rate in *Campylobacter*.

(Lin *et al*., 2003). Thus, this efflux pump has an important natural function in addition to conferring antibiotic resistance.

Another mechanism utilized by *Campylobacter* for antibiotic resistance is target modification. Spontaneous point mutations in the quinolone resistance determining region of GyrA reduce the affinity of fluoroquinolones (FQs) to DNA gyrase and result in resistance to this class of antibiotics (Engberg *et al*., 2001). Likewise, certain mutations in the 23S rRNA subunit mediate resistance to macrolide antibiotics (Gibreel *et al*., 2005). Due to the clinical importance of FQs and macrolides in treating campylobacteriosis, the rising prevalence of resistance to these two classes of antibiotics, especially to FQs, has become a concern for public health. *Campylobacter* has a high mutation frequency (up to 10^{-6}) for FQ resistance, which is affected by the function of Mfd (Yan *et al*., 2006; Han *et al*., 2008). Mfd is a transcription–repair coupling factor involved in strand-specific DNA repair (Selby and Sancar, 1994). In contrast to its mutation-frequency-decline function in *E. coli*, the Mfd orthologue in *C. jejuni* actually promotes the emergence of spontaneous FQ-resistant mutants in *Campylobacter* (Han *et al*., 2008). Knocking out the *mfd* gene in *Campylobacter* resulted in a 100-fold reduction in the number of spontaneous mutants resistant to ciprofloxacin, while overexpression of *mfd* increased the mutant numbers, suggesting that Mfd modulates the spontaneous mutation rates in *Campylobacter*. Due to the high spontaneous mutation rate and adaptive response in gene expression, FQ-resistant *Campylobacter* rapidly emerge from a FQ-susceptible population when exposed to FQ antibiotics. This has been shown by both *in vitro* and *in vivo* treatment studies (McDermott *et al*., 2002; Luo *et al*., 2003; Han *et al*., 2008) and represents a distinct feature of FQ-resistance development in *Campylobacter*. The resistance-conferring mutation in GyrA not only affects the susceptibility of *Campylobacter* to FQ antibiotics, but also modulates the fitness of this organism because FQ-resistant *Campylobacter* can out-compete FQ-susceptible *Campylobacter* in the absence of antibiotic selection pressure (Luo *et al*., 2005). In contrast to FQ resistance, the spontaneous mutation rate to macrolide resistance is low ($< 10^{-9}$), and the development of stable macrolide-resistant mutants appears to require long-term exposure to the antibiotics (Lin *et al*., 2007; Luangtongkum *et al*., 2009), which explains, at least partly, why the overall prevalence of macrolide-resistant *Campylobacter* is lower than that of FQ-resistant *Campylobacter*.

Biotechnological approaches for control of *Campylobacter*

Control of *Campylobacter* represents a major goal for improving food safety and public health. As *Campylobacter* is a foodborne pathogen of animal origin and is transmitted to humans through the food chain, intervention strategies should consider the ecological aspects of the organism and can be designed to target the pathogen in both animal and human hosts, as well as the different segments of food production systems. Given the fact that contaminated poultry meat is a major source of human infections with *Campylobacter*, reduction of this pathogen in commercial chickens both at the pre-slaughter and post-slaughter stages has been a focus of investigation. A quantitative risk assessment estimated that a two-log reduction in the number of *Campylobacter* on chicken carcasses could lead to a 30-fold reduction in the incidence of human campylobacteriosis (Rosenquist *et al*., 2003).

Bacteriophage therapy has received considerable attention lately. Administering lytic bacteriophages to artificially contaminated chicken carcasses or *Campylobacter*-colonized chickens reduced the level of *C. jejuni* contamination or colonization with varying success (Atterbury *et al*., 2003; Loc *et al*., 2005; Wagenaar *et al*., 2005). The efficacy of phage therapy is affected by phage types and treatment doses (Loc *et al*., 2005). In addition, treatment of *Campylobacter* colonization with bacteriophages was shown to be effective only for a short period, presumably due to development of phage resistance (Loc *et al*., 2005; Wagenaar *et al*., 2005). One potential way to avoid this limitation is to apply this treatment to chickens in the final days preceding slaughter, which may effectively reduce the quantity of *Campylobacter* that are introduced to the abattoir and consequently decrease chicken carcass contamination.

Recent advances in the field of metagenomics present a novel approach to the rational design of competitive exclusion products to potentially reduce *Campylobacter* colonization. Previously, competitive exclusions were attempted using culture-based, mucous-associated bacteria and yielded insufficient protection and inconsistent results (Schoeni and Doyle, 1992; Schoeni and Wong, 1994). The culture-based method potentially misses key constituents, such as fungi, anaerobes or fastidious microbes, resulting in non-reproducible effects of this treatment approach. The recently developed molecular techniques, such as oligonucleotide fingerprinting of rDNA genes (OFRG) (Patton *et al*., 2008) and high-throughput pyrosequencing of rDNA genes (Qu *et al*., 2008), provide powerful tools to assess the microbial ecology of the gut. Application of OFRG, in conjunction with antibiotic dissection of the microbial community in the turkey ceca, revealed a possible association between the presence of a subspecies of *Megamonas hypermegale* and *Campylobacter* suppression (A. Scupham, pers. comm.). As technology and data analysis methods advance, it is possible that more effective competitive

exclusion products can be developed to control *Campylobacter* in animal reservoirs.

Bacteriocins are ribosomally synthesized peptides produced by bacteria that inhibit the growth of other bacteria (Cotter *et al*., 2005). Several bacteriocins, notably bacteriocin OR-7 from *Lactobacillus salivarius* (Stern *et al*., 2006) and E-760 from *Enterococcus* (Line *et al*., 2008), demonstrated significant antagonistic effects on *Campylobacter*. Administration of either bacteriocin to chickens reduced the colonization level of *Campylobacter* by more than 10^6 colony-forming units per gram of cecal contents (Stern *et al*., 2006; Line *et al*., 2008). These results suggest that bacteriocin treatment is a promising approach for the control of *Campylobacter*. When used prior to slaughter, this strategy may be effective in reducing chicken carcass contamination by *Campylobacter*.

Vaccines may be designed to prevent *Campylobacter* infection in humans or in the chicken host. Various approaches for immunization against *Campylobacter* colonization in chickens have been discussed in a recent review (de Zoete *et al*., 2007). Whole-cell vaccines appear to confer marginal protection in chickens (Noor *et al*., 1995; Rice *et al*., 1997). Heterologous expression of *Campylobacter* antigens in an attenuated *Salmonella* strain has been used as a potential vector vaccine for poultry immunization, and this approach was met with varying success (Wyszynska *et al*., 2004; Sizemore *et al*., 2006). Surface-exposed proteins, such as flagellin, MOMP (the major outer membrane protein) and adhesins (e.g. PEB1), may serve as potential subunit vaccine candidates. For example, vaccination of mice with recombinant FlaA or PEB1 demonstrated partial protection against subsequent *C. jejuni* challenge (Lee *et al*., 1999; Du *et al*., 2008). A CPS conjugate vaccine of *C. jejuni* 81-176 provided complete protection against diarrhoea from homologous challenge, but did not prevent colonization in the New World monkey (Monteiro *et al*., 2008). Alternatively, secreted proteins can also be exploited as vaccine candidates. The flagella apparatus is known to secrete virulence factors (e.g. FlaC, Cia, FspA1 and FspA2), and vaccination of mice with recombinant FlaC, FspA1 and FspA2 elicited a humoral response and yielded partial protection against homologous challenge (Baqar *et al*., 2008). Despite these studies, an effective vaccine against *Campylobacter* has yet to be developed. As *Campylobacter* strains are genetically and antigenically diverse, an ideal vaccine should have the ability to elicit a protective immunity against a broad range of *Campylobacter* subtypes. Proteomics provides a promising tool for global profiling of *Campylobacter* antigens (Scott and Cordwell, 2009). Combination of proteomics with immunoblotting may identify protective antigens for vaccine development.

The distinct features of *Campylobacter* biology provide potential targets for the development of new antimicrobials or alternatives to antibiotics. *Campylobacter* preferentially uses flavodoxins as electron carriers and is predicted to synthesize menaquinone through the futalosine pathway (Cremades *et al*., 2005; Hiratsuka *et al*., 2008). The lack of flavodoxins and the futalosine pathway in humans and their important role in *C. jejuni* metabolism make them attractive therapeutic targets. As CmeABC is a key player in the resistance to antibiotics and in intestinal colonization, inhibition of CmeABC represents a plausible approach for controlling *Campylobacter*. A few studies have explored this possibility using efflux pump inhibitors, such as phenyl-arginine-β-naphthylamide (PAβN) and 1-(1-naphthylmethyl)-piperazine (NMP), which reduced *Campylobacter* resistance to antibiotics and bile salts in culture media (Lin and Martinez, 2006; Gibreel *et al*., 2007; Hannula and Hanninen, 2008). However, the tested inhibitors including PAβN had a limited effect on *Campylobacter* colonization in chickens (Lin and Martinez, 2006). To date, CmeABC-specific inhibitors have not been identified. Elucidation of the three-dimensional structure of the efflux proteins may facilitate the design of small molecular blockers that can be used to inhibit the function of CmeABC. Recently, Jeon and Zhang (2009) examined the feasibility of using antisense technology to silence the function of CmeABC and found that a CmeA-specific peptide nucleic acid sensitized *C. jejuni* to antibiotics. Whether this method can be optimized for *in vivo* use in preventing *Campylobacter* colonization awaits further investigation. To be effective *in vivo*, efflux pump inhibitors must be stable in the gastrointestinal tract, have a low toxicity to animal hosts and specifically target *Campylobacter* without inhibiting the normal gut flora.

In summary, the recent advances in understanding *Campylobacter* biology have provided us with new opportunities to develop anti-*Campylobacter* strategies. As research efforts expand from the genome to the transcriptome, proteome, glycome, metabolome and metagenome, we will be better equipped with biotechnological tools to control *Campylobacter* infection in both animal reservoirs and humans. In addition, the biological systems in *Campylobacter* may be exploited for broad biotechnological applications. For example, the *N*-glycosylation system in *C. jejuni* may be used to produce glycosylated recombinant proteins because this system is functional in *E. coli* (Wacker *et al*., 2002). The PglB enzyme of the *C. jejuni* glycosylation pathway was also shown to be able to transfer the *O*-polysaccharide from a lipid carrier to an acceptor protein in *E. coli* and *Salmonella* (Feldman *et al*., 2005; Wacker *et al*., 2006). The functionality of the *Campylobacter* glycosylation system in heterologous bacterial hosts and its relaxed substrate specificity provide a

suitable system to engineer glycoproteins for various biotechnological applications, such as production of glycoconjugate vaccines.

Acknowledgement

The work conducted in Zhang's laboratory is supported by National Institute Health Grant RO1DK063008 and National Research Initiative Competitive grants 2005-51110-03273 and 2006-34211-17310 from the US Department of Agriculture Cooperative State Research, Education, and Extension Services.

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