Comparative *in silico* analysis of chemotaxis system of *Campylobacter fetus*.

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Running title: *C. fetus* chemotaxis pathway genes.

Abstract

Chemoreceptor and chemotaxis signal transduction cascade genes of *C. fetus* subsp. *fetus* 82-40 show high level of similarity to that in *C. jejuni* and appears to include sixteen diverse transducer-like protein (*tlp*) genes that appear similar to nine of the twelve *tlp* genes in the *C. jejuni* NCTC 11168 with a percent identity ranging from 15% to 50%. Sixteen putative *C. fetus* 82-40 *tlp* genes belong to three classes: A, B and C, as well as an aerotaxis gene, based on their predicted structure. *C. fetus fetus* 82-40 chemoreceptor and chemotaxis signal transduction pathway genes have close phylogenetic relationship of chemotaxis genes among *Campylobacteraceae* and *Helicobacteraceae*

Keywords: Chemotaxis; Chemosensory receptors; *Campylobacter fetus*

Introduction

The bacteria belonging to species *Campylobacter fetus* are recognized as an important animal and human pathogens (Vandamme et al. 1991). *C. fetus* can be divided into two subspecies, *C. fetus* subsp. *venerealis* (CFV) and *C. fetus* subsp. *fetus* (CFF) and includes an intermediate of *C. fetus* subsp. *venerealis*, designated biovar intermedius (CVI) (Veron and Chatelain, 1973). CFV shows a strong tropism for the bovine genital tract causing bovine genital campylobacteriosis (BGC), a disease that causes the destruction of the cattle's embryo early in gestation (Garcia et al. 1983). Human infections with this subspecies are not common (Thompson and Blaser 1995). CFF on the other hand can cause infections in humans and animals including, cattle, goats, pigs, horses, fowl and reptiles and can cause abortion in sheep and cattle (Dennis 1975; Harvey and Greenwood 1985; Garcia et al. 1983; Meinershagen et al. 1965; Tu et al. 2004; Watson et al. 1967).

Although the mechanisms of campylobacter pathogenicity are poorly understood, motility and chemotaxis have been shown to be important for the initiation of the disease process for *Campylobacter* species (Hugdahl et al. 1988; Ferrero and Lee 1988; Lee et al. 1986; Szymanski et al. 1995; Takata et al. 1992; Terry et al. 2005).

In silico analysis of the genome sequence of *C. jejuni* NCTC 11168 (Marchant et al. 2002), revealed that in *C. jejuni*, some of the key signal transduction chemotaxis proteins, such as CheW and CheY, are conserved, while others show differences to those found in *E. coli*. CheB protein lacks a CheY-like receiver domain while CheA protein contains an additional C-terminal domain homologous to CheY. *C. jejuni* also encodes a CheV protein, similar to CheV in *B. subtilis* (Fredrick and Helmann 1994) and a putative analogue of CheZ like *Helicobacter pylori* (Terry et al. 2006).

Ten chemoreceptor genes of *C. jejuni* NCTC 11168 (designated transducer-like proteins Tlps) and two aerotaxis gene homologs were structurally categorized into three

groups, A, B, and C (Marchant et al. 2002). To date there are seven group A sensory receptors have been described for *C. jejuni*: Tlp1, Tlp2, Tlp3, Tlp4, Tlp7, Tlp10 and Tlp11. It is interesting to note that although the DNA and amino acid sequences of the group A Tlps are highly conserved, the presence of these receptors in *C. jejuni* strains is highly variable with only Tlp1 and Tlp10 consistently present in all strains described to date (Korolik and Ketley, 2008). Due to the typical three-domain structure of the group A chemoreceptors (a variable periplasmic sensory domain and conserved transmembrane and cytoplasmic signaling domains), these receptors are considered to be responsible for sensing ligands external to the cell, similar to *E. coli* (Falke, 1997). It is yet to be established what role a particular subset of sensory receptors may play in colonization of animal hosts or in virulence of *C. jejuni* strains. To date the only receptor-ligand interaction for the Tlp1 (CcaA) aspartate sensory receptor, has been demonstrated (Hartley-Tassell et al. 2010). This receptor was also shown to signal through the CheV, rather than CheW in the sensory receptor complex. In addition, interactions were detected between the Tlps 4, 6 and 8 and CheV, via global two-hybrid analysis, however, in this instance, specific interactions were not further explored (de Rita). These data indicate that in *C. jejuni*, the sensory receptor complex, at least for periplasmic sensory receptors, uses CheV, and not CheW as scaffolding protein in signal transduction. Chemoreceptor group B contains one cytoplasmic receptor homologue (Tlp9 or CetA) and group C contains Tlps 5, 6, 8, that are considered to be receptor proteins able to detect physiological cytoplasmic signals (Marchant).

Chemosensory receptors of *C. fetus*.

Comparison of the published sequences of *C. jejuni* NCTC 11168 Groups A, B, C Tlps and aerotaxis receptor proteins with those encoded by the genomic sequence of *C. fetus* 82-40 (using the Clustal W, BioEdit sequence alignment editor and TMHMM2, based on Hidden Markov Model) revealed presence of putative orthologs of chemotaxis receptor

genes in *C. fetus* 82-40 genome. Sixteen *tlp*-like genes in *C. fetus* 82-40 genome appeared orthologous to nine of the twelve *tlp* genes in the *C. jejuni* NCTC 11168 with a percent identity ranging from 15% up to 50% (similarity range 33% to 72% - protein-protein comparison; Table 1). The full amino acid sequences of four *C. fetus* 82-40 putative Tlp proteins, CFF82-40-1041, CFF82-40-0185, CFF82-40-1645 and CFF8240-0065 showed 50%, 34%, 32% and 15% identity and 72%, 55%, 52% and 33% similarity respectively with the protein sequence from the same gene, *C. jejuni* NCTC11168-1506c (*tlp1*; Table 1). The highest percent identity 50% (similarity 72%), with *C. jejuni* NCTC11168-1506c *tlp1* was present in CFF82-40-1041 (Table 1). Similarly, four other *C. fetus* 82-40 putative chemoreceptor proteins; CFF82-40-1305, CFF82-40-0190, CFF82-40-1698 and CFF82-40-1307 showed highest level of similarity with a single *C. jejuni* Tlp protein, Tlp4 (NCTC11168-0262c), whereas genes CFF82-40-0511 and CFF82-40-0975 revealed similarity with *C. jejuni* Tlp6 (NCTC11168-Cj0448c; Table 1). Although CFF82-40-1041 showed the highest percent similarity with *C. jejuni* aspartate receptor Tlp1 (NCTC11168-1506c), and could be the putative *C. fetus* 82-40 *tlp1* ortholog, similarity levels do not exclude the possibility that either one of the three *C. fetus* 82-40 *tlp* genes, CFF8240-0185, CFF82-40-1645 and CFF82-40-0065 could instead be the ortholog of *C. jejuni* NCTC11168-1506c (*tlp1*; Table 1). Experimental evidence is necessary in order to verify the ligand binding specificity of each of these *C. fetus* 82-40 chemoreceptors. These data, never the less, allow to speculate that *C. fetus* may be able to respond to a wider range of ligands than *C. jejuni* or indeed may have some redundancy in receptor specificity, where two to four receptors may be able to sense the same ligand, perhaps providing signal amplification for detection of essential nutrients.

It should be noted that the manual scoring of pair-wise alignments for both full coding sequences and sensory domain coding sequences of the *C. fetus* 82-40 receptor genes with

their respective orthologs in the tested Epsilon proteobacteria genomes indicated an interesting discrepancy with BLASTp analysis. In the case of 11 of *C. fetus* 8240 *tlp* genes, their sequences revealed identity with the same corresponding *C. jejuni* NCTC11168 Tlp sequence, irrespective of whether the complete amino acid sequence was used for analysis, or only the sequence of the periplasmic sensory domain. However, five of the sixteen Tlp sequences (CFF82-40-0185, CFF82-40-1645, CFF82-40-1227, CFF82-40-0139 and CFF82-40-0065) aligned with a different protein of *C. jejuni* NCTC11168 when the sensory domain sequences only were compared (Table 2). When the periplasmic sensory domains were aligned to proteins encoded by the *C. jejuni* NCTC11168 genome, CFF82-40-1645, CFF82-40-1227 and CFF82-40-0065 no longer aligned with *C. jejuni* Tlps, instead aligning with an acid membrane protein, putative ATP/GTP binding protein and restriction modification enzyme respectively. This finding underlines the importance of manual and reciprocal sequence alignment analysis for maximum accuracy. It is particularly pertinent for genes with multiple domains, where some domains may be highly conserved due to their function, such as signalling domains of *tlp* genes and signalling proteins in general. In this case, the high level of similarity among signalling domains of chemosensory proteins, had obscured the fact that their sensory domains, which determine receptor-ligand specificity, were each similar to a different receptor or another protein with lower overall gene similarity.

Comparison of *C. fetus* 82-40 chemosensory receptors with the genomes of other Epsilon proteobacteria as well as with two representative genomes of the family *Helicobacteraceae*; *H. pylori* 26695 and *W. succinogenes* DSM 1740 (Table 3) showed that percent identity of *C. fetus* 82-40 *tlp* genes with their orthologs in all the examined genomes is in the range of 12% to 72% (similarity range 32% to 86% - protein-protein comparison, Table 3). Conversely, the periplasmic sensory domains of the Tlps show high degree of

interspecies and intraspecies variability, necessitating independent comparisons of all group A *tlp* genes sensory domains as was performed for *C. jejuni*.

Signal transduction pathway of *C. fetus*.

Interestingly, similar to that in *C. jejuni* NCTC11168 genome (Parkhill et al. 2000), most of the *tlp* genes as well as two of the chemotaxis pathway genes; *cheZ* analogue and the RR *cheY* in *C. fetus* 82-40 genome were scattered in the genome and three chemotaxis genes *cheA*, *cheW* and *cheV*, as well as *cheR*, *cheB* pair, are contiguous. The comparative *in silico* analysis revealed that *C. fetus* 82-40 chemotaxis signal transduction pathway genes (*cheA*, *cheW*, *cheY*, *cheV*, *cheB* *cheR* and *cheZ* orthologs) had the expected high level of similarity with their corresponding orthologs in the *C. jejuni* NCTC11168 genome (Parkhill et al. 2000), for example CFF82-40-0331 gene (*cheA*) showed 79% identity (91% similarity – protein-protein comparison) with Cj11168-0284c (*cheA*) (Table 1).

C. fetus 82-40 signal transduction genes are also similar to their respective orthologs in genomes of Epsilon proteobacteria, *C. jejuni* 84-25 (JCVI-CMR), *C. coli* RM2228, *C. lari* RM2100, *C. upsaliensis* RM3195 (Fouts et al. 2005) and the related Epsilon proteobacteria *H. pylori* 26695 (Tomb et al. 1997) and *W. succinogenes* DSM 1740 (Baar et al. 2003). All of the signal transduction genes orthologs were highly conserved in the examined genomes of *C. jejuni* 84-25, *C. coli* RM2228, *C. lari* RM2100, *C. upsaliensis* RM3195 and *W. succinogenes* DSM 1740 (Table 1 and 3), indicating functional conservation of the chemotaxis signal transduction cascade in Epsilon proteobacteria.

Phylogenetic relationships of chemosensory receptors of *C. fetus* with the Epsilon proteobacteria chemosensory receptors.

Close phylogenetic relationships exist between the sixteen *C. fetus* 82-40 *tlp* genes with their corresponding orthologs in the genomes of *Campylobacteraceae*, (*C. jejuni*

NCTC11168, *C. jejuni* 84-25, *C. coli* RM2228, *C. lari* RM2100, *C. upsaliensis* RM3195) and *Helicobacteraceae* (*H. pylori* 26696, *W. succinogenes* DSM 1740), where clusters with a bootstrap value greater than or equal to 75% were defined as confirmed subgroups (Tripathi and Sowdhamini 2008). Unexpectedly, clustering patterns of the sixteen *C. fetus* 82-40 *tlp* genes were different. Most *C. fetus* Tlps clustered with the other *Campylobacteraceae* in various relationships (Figure 1a and b for examples; Figures S1-3), however, some of the receptors clustered closer to *W. succinogenes* DSM 1740 (Figure 1c for example; Figure S4). Three of the *C. fetus* *tlp* genes, CFF8240-1041, CFF8240-1645 and CFF8240-0322, clustered with their respective orthologues in both *C. jejuni* NCTC11168 and *C. jejuni* 8425, with 97%, 86% and 79% bootstrap values respectively (Figure 1 and Figure S1). CFF8240-0065 *tlp* gene fell into a cluster with *C. upsaliensis* RM3195 CUP-1515 and *C. coli* RM2228 CCO-0518 (Figure S2) and one of the sixteen *C. fetus* 8240 *tlp* genes, CFF8240-0139, grouped with *W. succinogenes* DSM 1740 WS-1861, with *C. coli* RM2228 CCO-1600 and *C. jejuni* NCTC11168-1492c with bootstrap support of 93% (Figure S3). Receptor gene CFF8240-1223, clustered with its corresponding orthologue in *C. lari* RM2100 CLA-0003 with 78% bootstrap support (Figure S4) and CFF8240-1227 clustered with two of its corresponding orthologues in both *C. lari* RM2100 CLA-0183 and *W. succinogenes* DSM 1740 WS-0843 with 53% bootstrap support (Figure 1). It is also interesting to note that *C. fetus* *tlp* 8240 genes CFF8240-0511 clustered with *W. succinogenes* DSM 1740 WS-0128 and WS-0696 with 100% and 85% bootstrap support (Figure 1c).

Phylogenetic analysis of the *C. fetus* 82-40 *tlp* and chemotaxis pathway protein sequences has consequently identified seven different phylogenetic relationships for *C. fetus* 82-40 genes. *C. fetus* chemosensory genes. Unfortunately, until the ligand specificity of each of the chemosensory receptors is identified, it is difficult to explain why *C. fetus* genome encodes

such diverse range of sensory receptors. This underlines the fact that it is not possible to assign function to chemosensory receptor homologue or orthologue genes based on sequence similarities and that experimental data is required to determine the specificity of receptor-ligand interactions. Recently identified *C. jejuni* aspartate receptor (Tlp1), with 0% similarity to the sensory domain amino acid sequence to the well characterised *E. coli* aspartate receptor (Hartley) provides an excellent example of proteins with different amino acid composition forming a similar tertiary structure able to bind the same ligand. The diversity of the *C. fetus* sensory receptor gene set appears to be an excellent example of the mosaic nature of bacterial genomes where different sensory genes were likely to have been collected over time from many related organisms to allow survival in various environmental niches and to move to different hosts to expand the host range. The apparent diversity of *C. fetus* chemosensory genes could allow for the differential tissue tropism of this bacteria being able to colonise/infect the animal intestinal tract as well as the genital tract of cattle.

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Table 1. Comparison of sequence identity and similarity of the chemoreceptor and chemotaxis pathway genes in *C. fetus* 82-40, *C. jejuni* NCTC 11168 and *C. jejuni* 84-25

Chemotaxis genes	<i>C. fetus</i> 82-40 CFF82-40	<i>C. jejuni</i> NCTC 11168 Cj11168	% I/S	<i>C. jejuni</i> 84-25 CJJ84-25	% I/S
Group_A	1041	1506c (tlp1)	50/72	1589	53/75
	1305	0262 (tlp4)	40/64	0173	38/62
	0190	0262 (tlp4)	41/65	0173	37/60
	0562	0952 (tlp7)	44/70	0982	44/71
	1698	0262 (tlp4)	40/65	0173	38/61
	0185	1506c (tlp1)	34/55	1589	32/52
	1645	1506c (tlp1)	32/52	1589	42/62
	1227	1564 (tlp3)	29/51	0173	29/51
	0139	1492c	18/37	0173	17/38
	0065	1506c (tlp1)	15/33	1589	14/32
	1307	0262 (tlp4)	40/65	0173	36/58
Group_A/B	1223	0019c (tlp10)	22/50	0043	20/46
Group_C	0975	0448c (tlp6)	28/56	0472	47/69
	0322	1110c (tlp8)	77/89	1138	78/89
	0511	0448c (tlp6)	36/62	0472	22/36
Aer	1012	1189 ¹	44/64	1214	43/64
<i>cheW</i>	0332	0283c	41/68	0307	69/84
<i>cheA</i>	0331	0284c	79/91	0308	79/90
<i>cheY</i>	1193	0643 ²	41/68	0676	41/68
<i>cheB</i>	0805	0924c	48/73	0946	72/48
<i>cheR</i>	0803	0923c	43/66	0945	43/65
<i>cheV</i>	0330	0285c	70/88	0309	87/69
<i>cheZ</i> H⁴	1051	0700 ³	57/75	0732 ^c	87/69

¹: cetB, ²: cbrR (Campylobacter bile resistance Regulator) protein, a two-component response regulator,

³:hypothetical protein, ⁴:H:homolog, %I/S Identical / Similar amino acids.

Table 2 Five putative *C. fetus* tlp genes (full coding region and sensory domain (SD) with their corresponding orthologous *C. jejuni* NCTC 11168 genes

Serial	<i>C. fetus</i> 82-40 CFF82-40	<i>C. jejuni</i> NCTC 11168 Cj11168	%I/S
1	0185	1506c (tlp1)	34/55
	SD	1506c (tlp1) SD	11/24
	SD	1564 (tlp3)	11/ 27
2	1645	1506c (tlp1)	32/52
	SD	1506c (tlp1) SD	12/29
	SD	1363 ^a	24/49
3	1227	1564 (tlp3)	29/51
	SD	1564 (tlp3) SD	11/25
	SD	0412 ^b	24/46
4	0139	1492c	18/37
	SD	1492c_no_SD	N/A
		0144	17/37
5	0065	1506c (tlp1)	15/33
	SD	1506c (tlp1) SD	17/29
	SD	1051c ^c	27/47

^a: acid membrane antigen A, ^b: putative ATP/GTP binding protein, ^c: restriction modification enzyme *cjeI*. N/A:

not applicable, %I/S Identical / Similar amino acids.

Table 3. Comparative analysis of sequence identity and similarity of chemoreceptor and chemotaxis pathway genes in *C. fetus* 82-40, *C. coli* RM2228, *C. lari* RM2100 and *C. upsaliensis* RM3195, *H. pylori* 26695 and *W. succinogenes* DSM 1740

Chemotaxis genes	<i>C. fetus</i> 82-40 CFF82-40	<i>C. coli</i> RM2228 CCO	%I/S	<i>C. lari</i> RM2100 CLA	%I/S	<i>C. upsaliensis</i> RM3195 CUP	%I/S	<i>H. pylori</i> 26695 HP	%I/S	<i>W. succinogenes</i> DSM 1740 WS	%I/S
Group _A	1041	0334 (tlpA)	52/77	0356 (tlpA)	38/58	1752 (tlpA)	45/67	0099 (tlpA)	31/56	0864	33/58
	1305	1678 (tlpA)	39/62	0135 (tlpA)	39/64	1790 (tlpA)	40/62	0082 (tlpC)	30/59	1333	43/69
	0190	1678 (tlpA)	38/60	0135 (tlpA)	41/66	1124 (tlpA)	40/62	0082 (tlpC)	29/57	1333	34/56
	0562	0943 ^a	36/68	0908 (tlpB)	40/71	0527 ^f	39/64	0103 (tlpB) ^h	21/48	2114	30/62
	1698	1678 (tlpA)	37/58	0135 (tlpA)	41/67	1124 (tlpA)	39/61	0099 (tlpA)	30/55	1333	33/54
	0185	1678 (tlpA)	32/52	0356 (tlpA)	34/52	1790 (tlpA)	34/52	0099 (tlpA)	29/49	1333	37/58
	1645	1678 (tlpA)	36/54	0356 (tlpA)	35/54	1124 (tlpA)	34/53	0099 (tlpA)	28/50	1333	35/59
	1227	0334 (tlpA)	29/48	0183	26/46	1790 (tlpA)	30/51	0099 (tlpA)	26/53	0843	37/64
	0139	1600	17/36	0135 (tlpA)	16/38	1790 (tlpA)	14/33	0082 (tlpC)	12/32	1861 ^k	17/36
	0065	0518	40/68	0962	14/36	1515 ^g	42/68	0099 (tlpA)	14/34	0859	43/68
	1307	1678 (tlpA)	38/61	0135 (tlpA)	39/61	1790 (tlpA)	41/63	0099 (tlpA)	30/53	1333	38/61
	1041_SD	0334 (tlpA)	32/57	0356 (tlpA)	16/34	1752 (tlpA)	34/59	0099 (tlpA)	14/37	0864	15/35
	1305_SD	1678 (tlpA)	20/47	0135 (tlpA)	23/47	1790 (tlpA)	22/44	0082 (tlpC)	17/37	1333	17/36
	0190_SD	1678 (tlpA)	26/55	0135 (tlpA)	24/52	1124 (tlpA)	21/43	0082 (tlpC)	13/36	1333	17/39
	0562_SD	0943a	36/71	0908 (tlpB)	39/66	0527b	38/66	0103 (tlpBh)	7/29	2114	4/6
	1698_SD	1678 (tlpA)	20/43	0135 (tlpA)	24/52	1124 (tlpA)	18/43	0099 (tlpA)	13/35	1333	17/36
	0185_SD	1678 (tlpA)	11/27	0356 (tlpA)	16/31	1790 (tlpA)	12/21	0099 (tlpA)	12/27	1333	16/36
	1645_SD	1678 (tlpA)	13/29	0356 (tlpA)	16/32	1124 (tlpA)	10/22	0099 (tlpA)	14/27	1333	14/31
	1227_SD	0334 (tlpA)	16/30	0183	10/23	1790 (tlpA)	14/29	0099 (tlpA)	11/26	0843	13/36
	0139_SD	1600	12/32	0135 (tlpA)	19/44	1790 (tlpA)	14/33	0082 (tlpC)	14/37	1861d	12/25
	0065_SD	0518	38/65	0962	15/26	1515c	39/60	0099 (tlpA)	11/26	0859	46/71
	1307preTMM	1678 (tlpA)	17/44	0135 (tlpA)	23/50	1790 (tlpA)	15/31	0099 (tlpA)	13/32	1333	25/57
	1307postTMM		55/75		55/73		56/75		45/70		52/73
Aer	1012	1259	46/67	1341	41/65	0045	42/67	0621 ^j	19/44	0696	50/71
cheW	0332	0361 ^b	71/85	0319	70/81	0792	67/81	0391	53/79	2082	58/79
cheA	0331	0362 ^b	80/90	0318	79/89	0793	78/89	0392	79/89	2083	69/81
cheY	1193	0723 ^b	38/65	1020	39/66	0459	39/65	1067	11/19	0415	64/86
cheB	0805	1025 ^b	53/75	1051 ^e	46/70	0824	49/73	0568	15/31	1213	52/70
cheR	0803	1024 ^b	36/63	1052 ^f	42/70	0825	39/65	0664	14/30	1212	
cheV	0330	0363 ^b	68/87	0317	70/87	0794	70/87	0393	47/69	2084	
cheZ_H	1051	0766 ^c	59/74	1384 ^c	50/62	0560 ^c	56/73	0170	43/65	2123 ^c	

^a: probable membrane protein Cj0952, ^b: CCo is used as the annotation for chemotactic proteins, ^c: conserved hypothetical protein; ^d: MCP transducer/ sensor y box protein; ^e: putative CheB; f: putative CheR, ^f: carbamoyl-phosphate synthase, large subunit (carB); ^g: putative MCP, ^h: MCP-like protein *Helicobacter pylori* P12; ⁱ: Hemolysin secretion protein precursor; ^j: recombination and DNA strand exchange inhibitor protein or DNA mismatch repair protein (MutS); ^k: sensor kinase _RHPS. %I/S Identical / Similar amino acids.

Fig (1) Dendrogram based on alignment of *C. fetus* 82-40 Tlp protein CFF82-40-0190 (a), CFF82-40-1041 (b) and CFF82-40-1227 (c) with their respective orthologs in *C. jejuni* NCTC 11168, *C. jejuni* 84-25, *C. coli* RM2228, *C. lari* RM2100, *C. upsaliensis* RM3195, *H. pylori* 26696 and *W. succinogenes* DSM 1740.