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ORIGINAL PAPER

Molecular identification and characterization of clustered regularly interspaced short palindromic repeats (CRISPRs) in a urease-positive thermophilic *Campylobacter* sp. (UPTC)

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Abstract Novel clustered regularly-interspaced short palindromic repeats (CRISPRs) locus [7,500 base pairs (bp) in length] occurred in the urease-positive thermophilic *Campylobacter* (UPTC) Japanese isolate, CF89-12. The 7,500 bp gene loci consisted of the 5′-methylaminomethyl-2-thiouridylate methyltransferase gene, putative (P) CRISPR associated (p-*Cas*), putative open reading frames, *Cas1* and *Cas2*, leader sequence region (146 bp), 12 CRISPRs consensus sequence repeats (each 36 bp) separated by a non-repetitive unique spacer region of similar length (26–31 bp) and the phosphatidyl glycerophosphatase A gene. When the CRISPRs loci in the UPTC CF89-12 and five *C. jejuni* isolates were compared with one another, these six isolates contained p-*Cas*, *Cas1* and *Cas2* within the loci. Four to 12 CRISPRs consensus sequence repeats separated by a non-repetitive unique spacer region occurred in six isolates and the nucleotide sequences of those repeats gave approximately 92–100% similarity with each other. However, no sequence similarity occurred in the unique spacer regions among these isolates. The putative σ^{70} transcriptional promoter and the hypothetical ρ-independent terminator structures for the CRISPRs and *Cas* were detected. No in vivo transcription of p-*Cas*, *Cas1* and *Cas2* was confirmed in the UPTC cells.

Keywords *Cas* · CRISPRs · RT-PCR · Sequence analysis · UPTC

E. Tasaki and J. Hirayama contributed equally to this study and hence should be considered as equal first authors.

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Introduction

Clustered regularly-interspaced short palindromic repeats (CRISPRs) are a family of short and highly conserved DNA sequence repeats that have been found in many bacteria (Jansen et al. 2002; Mojica et al. 2005). In addition, the existence of several conserved CRISPR-associated (*Cas*) genes in the vicinity of CRISPR loci are often described (Godde and Bickerton 2006; Haft et al. 2005; Jansen et al. 2002). Regarding CRISPRs and *Cas*, it was recently proposed that CRISPR and *Cas* genes might be involved in confering immunity to the host cell against foreign DNA (Makarova et al. 2006; Mojica et al. 2005). In addition, regarding the spacers, Bolotin et al. (2005) recently suggested that the unique spacer elements were the traces of past invasions by extrachromosomal elements (Bolotin et al. 2005).

Most recently, regarding *Escherichia coli* CRISPR-*Cas* promoters and their silencing, Pul et al. (2010) demonstrated that DNA-binding protein H-NS is involved in silencing of the CRISPR-*Cas* promoters, resulting in cryptic Cas protein expression (Pul et al. 2010).

Campylobacter organisms, primarily *C. jejuni, C. coli* and *C. fetus* are Gram-negative bacteria, that are the major and typically recongnized *Campylobacter* organisms of medical, public health or veterinary interest worldwide (Debruyne et al. 2009; Lastovica and Skirrow 2000; Moore et al. 2005).

The thermophilic species *Campylobacter lari* was first isolated particularly from seagulls of the genus *Larus*

(Benjamin et al. 1983; Skirrow and Benjamin 1980). *C. lari* has also been shown occasionally to be a cause of clinical infection (Martinot et al. 2001; Nachamkin et al. 1984; Werno et al. 2002). In addition, an atypical group of isolates of urease-positive thermophilic *Campylobacter* sp. (UPTC) was isolated from the natural environment in England in 1985 (Bolton et al. 1985). Thereafter, these organisms have been described as a biovar or variant of *C. lari* (Mégraud et al. 1988; Owen et al. 1988). Subsequent isolates were obtained in France (Bezian et al. 1990; Mégraud et al. 1988), Northern Ireland (Kaneko et al. 1999; Matsuda et al. 2003; Wilson and Moore 1996), The Netherlands (Endtz et al. 1997) and Japan (Matsuda et al. 1996; Matsuda et al. 2002). Thus, these two representative taxa, namely urease-negative (UN) *C. lari* and UPTC occur within the species of *C. lari* (Matsuda and Moore 2004).

Regarding CRISPR and *Cas* in *Campylobacter* organisms, Schouls et al. (2003) employed sequence analysis of the CRISPRs to genotype a collection of *Campylobacter* strains (n = 180 for *C. jejuni*; n = 4 for *C. coli*). In addition, Price et al. (2007) described a novel method for genotyping the CRISPR locus of *C. jejuni* and *C. coli* (a total of 210 Australian isolates) subjected to high-resolution melt analysis following real-time PCR. However, no reports have appeared on CRISPRs and *Cas* with other *Campylobacter* organisms.

Although CRISPR and *Cas* have recently been identified in *C. fetus* subsp. *fetus* 82-40 (DDBJ/EMBL/GenBank accession number NC_008599), following whole genome shotgun sequencing analysis, reports have not yet appeared for *C. fetus.* In addition, the *C. lari* RM2100 strain, whose genome analysis has already been carried out (NC_012039; Miller et al. 2008), was shown not to carry any CRISPRs and *Cas*.

However, during the process of our genome sequence analysis for a representative taxon of *C. lari* UPTC isolate, we, for the first time, found the occurrence of the CRISPR and *Cas* in the environmental Japanese UPTC isolate CF89-12 genome DNA. Therefore, the aim of the present study was firstly to identify and molecularly characterize the CRISPR and *Cas* from the *C. lari* taxon, UPTC CF89-12 isolate. Moreover, we wished to clarify whether these genes are expressed in UPTC cells or not.

Materials and methods

Representative *C. lari* taxon, UPTC isolate employed in the present study and growth conditions

The Japanese isolate UPTC CF89-12, which was isolated from the water of Asahigawa River, Okayama prefecture, Japan (Matsuda et al. 1996), was employed in the present study.

Following culturing on the charcoal-cefazolin-sodium deoxycholate agar medium (Oxoid, Hampshire, UK), UPTC cells were cultured on blood agar base No. 2 (Oxoid) that contained defibrinated horse blood [7% (v/v); Nippon Bio-test, Tokyo, Japan], supplemented with Butzler *Campylobacter*-selective medium (Virion, Zurich, Switzerland), under microaerophilic conditions using BBL Campypak Microaerophilic System Envelopes (Becton–Dickinson, NJ, USA) at 37°C for 48 h. Cells were further cultured on Mueller–Hinton agar under the same microaerophilic conditions.

Genomic DNA preparation

UPTC CF89-12 genomic DNA was prepared by the cetyltrimethylammonium bromide (CTAB) method (Sambrook and Russell 2001) and following RNase treatment. The DNA concentration was adjusted to approximately 800 ng/μL.

Construction of the genome DNA library of UPTC CF89-12

A genomic DNA library was constructed using NEBNext™ DNA Sample Prep. Reagent Set 1 (New England BioLabs Japan Inc., Tokyo, Japan). The DNA was fragmented using Covaris S-series (Covaris Inc., MA, USA) and was separated by agarose gel electrophoresis [300–500 base pairs (bp)]. Cluster generation was carried out using the constructed library DNA as templates with Cluster Station and Cluster Generation Kit (Illumina Inc., Ca, USA).

Nucleotide sequence analysis

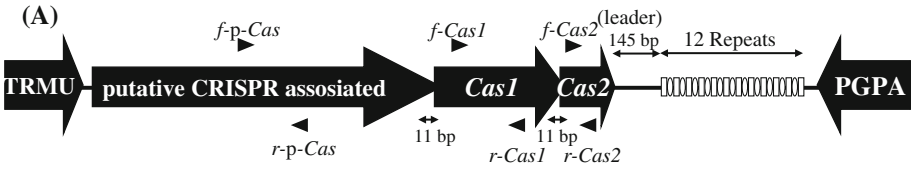
The nucleotide sequence was determined using Genome Analyzer IIx and Sequencing Kit (Illumina Inc.). Nucleotide sequence analysis of full-length CRISPRs locus was carried out using the GENETYX-Windows computer software version 9 (GENETYX Co., Tokyo, Japan).

Schematic representation of the CRISPRs locus in the UPTC CF89-12 isolate

CRISPRs loci for the UPTC CF89-12 organism (AB598370) analysed in the present study are illustrated in Fig. 1.

Total cellular RNA purification and reverse transcription (RT)-PCR

Total cellular RNA was extracted and purified from UPTC CF89-12 cells, using RNAprotect Bacteria Reagent and



(B)

Primer	Nucleotide sequence (5'→3')
f-p-*Cas* UPTC	GCKATWARWGARTATAGAAAGGTC
r-p-*Cas* UPTC	GTCTTAAARCYGATGTAAGCATAC
f-Cas1 UPTC	ACYTGYGATGAAWCTCAYARTATAAATGG
r-Cas1 UPTC	CTAAAMACTTCTATCAAATCATCRCAAAG
f-Cas2 UPTC	GAAGATAAATTTATGMGHGTKYTWTTGATGTTTG
r-Cas2 UPTC	CWGTKATGRTTAAARCYCTTAYATTTCCATAAGG

Fig. 1 The resultant schematic representation of the CRISPR and *Cas* locus (7,500 bp) identified in the UPTC CF89-12 and the locations of the primer pairs for the RT-PCR amplifications (**A**), and the primer sequences employed (**B**)

RNeasy Mini Kit (QIAGEN, Tokyo, Japan). Firstly, RT-PCR analysis was carried out using the primer pair of *f-/r-*p-*Cas*UPTC for the putative (p)-*Cas*, as shown in Fig. 1, with the QIAGEN OneStep RT-PCR Kit (QIAGEN). In the present study, we designed a primer pair *in silico* for RT-PCR amplification of the CRISPRs p-*Cas* transcripts segment from the UPTCCF89-12 isolate, based on sequence information (Nakanishi et al. 2010). Then, we also designed two primer pairs of *f-/r-Cas1*UPTC and *f-/r-Cas2*UPTC for RT-PCR amplification of the transcript segment for the *Cas1* and *Cas2* (Fig. 1), respectively. These primer pairs were expected to generate RT-PCR products of the transcript segments of 700 bp with the *f-/r-*p-*Cas*UPTC, 470 bp with the *f-/r-Cas1*UPTC and 230 bp with the *f-/r-Cas2*UPTC, respectively. These three primer pairs sequences correspond to the nucleotide positions (np) 2,824 through 2,847 bp and np 3,535 through 3,512 bp for the *f-/r-*p-*Cas*UPTC, np 4,643 through 4,671 bp and np 5,084 through 5,112 bp for the *f-/r-Cas1*UPTC and np 5,331 through 5,364 bp and 5,562 through 5,529 bp for the *f-/r-Cas2*UPTC of the nucleotide sequence data of the CRISPRs genes cluster including adjacent genetic loci of the UPTC CF89-12 isolate (AB598370). In addition, another PCR primer pair (f-/r-MOMP-common) constructed for the amplification of the two major outer membrane protein (MOMP) genes, *PorA1* and *PorA2* from *C. lari* (Hirayama et al. 2010) for the total cellular RNA positive-control of the UPTC CF89-12. This primer pair was expected to generate RT-PCR product of the transcripts segment of approximately 1,200 bp. For primer design purposes nucleotide sequence alignment analysis was carried out by employing CLUSTAL W software (1.7 program) (Thompson et al. 1994) incorporated in the DDBJ.

Amplified RT-PCR products were separated by 1% (w/v) agarose gel electrophoresis in 0.5× TBE at 100 V and detected by staining with ethidium bromide.

Results

CRISPR locus identification and the resultant schematic representation

The novel CRISPR locus (7,500 bp) that we have identified in the present study, consisted of the p-*Cas* [structural gene 3,012 bp, 1,003 amino acids (aa), np of the structural gene 1,438–4,449 bp], *Cas1* (903 bp, 300 aa, np 4,436–5,338 bp), *Cas2* (426 bp, 141 aa, np 5,325–5,750 bp), leader sequence region (146 bp, np 5,751–5,896 bp) and 12 CRISPRs consensus sequence repeats (each 36 bp; 5′-ATTTTATC ATAAAGAAATTTAAAAAGAGACTAAAAC-3′), separated by non-repetitive unique spacer regions of similar length (29–31 bp), as shown in Fig. 2. Thus, the 12 CRISPRs consensus sequence repeats of each 36 bp demonstrated an identical nucleotide sequence in the UPTC CF89-12. In addition, as shown in Fig. 2, the 11 non-repetitive unique spacer regions contained distinctly different nucleotide sequences to each other (Fig. 2). The resultant schematic representation of the CRISPR and *Cas* loci (7,500 bp) identified in UPTC CF89-12, is illustrated in Fig. 1A.

Non-repetitive unique spacer region (5'→3')
1. TTCATCTCGTATAAATTTACGATCTTTAA
2. TCAAAGCACTCCTGCTAAAGTTGCTGCTAA
3. CAAAACACTCACGGCTTTTGGATAATGGCA
4. TGCCGTTGATTTGCATATTATTACCATTTG
5. TATATTAGAAGCTTTAAAAGTTAATTTATTA
6. ATCAATGCTTTTAATCAAATGGAACAAATC
7. CAAAATCCTGTTTTAAATCCTATTTGTATT
8. CAAGTTCCCTTATTACCACGAGGAAAAACG
9. ATGGTGGCTTATCATACCCAAGAAATTACA
10. ACAGATGTGATGAATATCGTCGTTCTTTTT
11. TGATTTGTATCTTTTGTGTTCCCATAATAA

Fig. 2 Nucleotide sequences of the 11 unique spacer regions in the UPTC CF89-12 CRISPRs. The 11 unique spacer regions were numbered (nos. 1–11) from 5′ to 3′ in the CRISPRs

Fig. 3 Nucleotide sequence analysis for the identification of the putative transcription promoter structures in UPTC CF89-12 CRISPRs

In addition, a possible overlap of 14 bp (np 4,436–4,449 bp) occurred between p-*Cas* and *Cas1* structural genes (AB598370). Another possible overlap of 14 bp (np 5,325–5,338 bp) was also seen between *Cas1* and *Cas2* structural genes.

Putative promoter and hypothetical intrinsic ρ-independent transcription terminator structures

Regarding the identification of the putative promoter structures for the CRISPRs and *Cas* loci, the authors attempted to perform nucleotide sequence alignment analysis of the 100 bp region upstream of the p-*Cas* ORFs among UPTC CF89-12 and five *C. jejuni* 81116, NCTC11168, RM1221, subsp. *doylei* 269.97, IA3902 isolates, (data not shown). The nucleotide sequence of UPTC CF89-12 is shown in Fig. 3. In this region, a typical promoter consensus sequence at -10 region (TATAAT) was seen at the locus between np 1,395 and 1,360 bp for UPTC CF89-12. However, no consensus sequences at the -35 region were identified, and a semi-conserved T-rich region (T, 11/17) was identified between np 1,354 and 1,370 bp, instead of the region, as shown in *Rpo*D promoters in the genome of *C. jejuni* (Petersen et al. 2003) (Fig. 3). In addition, similar promoter structures were also seen among the *five C. jejuni* isolates (data not shown). Therefore, these CRISPR and *Cas* genes may possibly be transcribed by the σ70 factor in the UPTC and *C. jejuni* organisms, as described by Petersen et al. (2003).

We also attempted to perform nucleotide sequence alignment analysis of the leader sequence region (np 5,751–5,896 bp) immediately upstream of the CRISPRs in order to identify any other promoter structure(s) which exist for the CRISPRs among the UPTC F89-12 and five *C. jejuni* isolates. However, no typical promoter consensus sequences or semi-conserved T-rich region were identified (data not shown).

Moreover, the hypothetically intrinsic ρ-independent transcription terminator structure which contains a G + C rich region near the base of the stem between np 6,886 and 6,901 bp and a single-stranded run of T residues (np 6,901–np 6,904) were seen downstream of the 12 CRISPRs consensus sequence repeats in UPTC CF89-12 (Fig. 4).

Regarding the regions immediately upstream of the p-*Cas* and downstream of the 12 CRISPRs consensus repeats within the 7,500 bp locus in the UPTC CF89-12 isolate, two putative and full-length structural genes

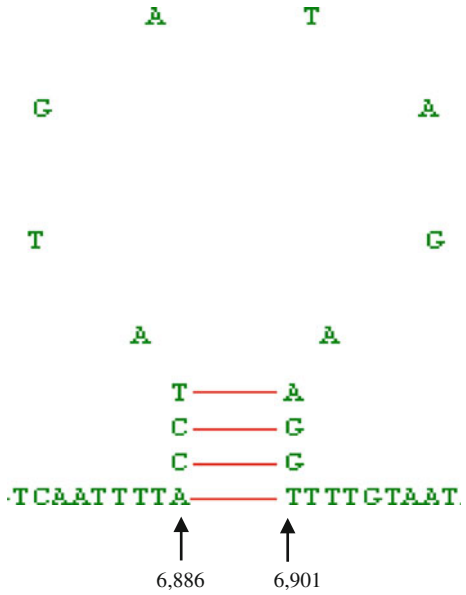


Fig. 4 Nucleotide sequence analysis for the identification of the putative intrinsic ρ-independent transcription terminator structure in the UPTC CF89-12 CRISPRs

of the 5′-methylaminomethyl-2-thiouridyl-ate methyltransferase (TRMU; structural gene 1,017 bp in length, np 179–1,195 bp) and the phosphatidyl glycerophosphatase A (PGPase, 489 bp, np 6,736–7,224 bp; reverse direction), respectively were identified.

In addition, the amino acid sequence of the putative ORF of TRMU and PGPase from UPTC CF89-12 gave so high a sequence similarity of approximately 94 and 96% to those two counterparts in the *C. lari* RM2100 strain (NC_012039, Miller et al. 2008), respectively. Thus, the CRISPRs and *Cas* locus (approximate 5,700 bp) within the UPTC CF89-12 isolate occurred between the TRMU and PGPase structural genes, which both gave high sequence similarities to those two counterparts in the *C. lari* RM2100 strain, as described above.

No in vivo transcription of the p-*Cas, Cas1* and *Cas 2* genes

When RT-PCRs were carried out with UPTC CF89-12 using the three primer pairs (*f-/r*-p-*Cas*UPTC for the p-*Cas*; *f-/r-Cas1*UPTC for *Cas1*; *f-/r-Cas2*UPTC for *Cas2*) to amplify the gene transcripts of the CRISPRs and *Cas* locus, no RT-PCR signals of expected sizes were seen (Fig. 5). However, in vivo MOMP transcription, employed as a

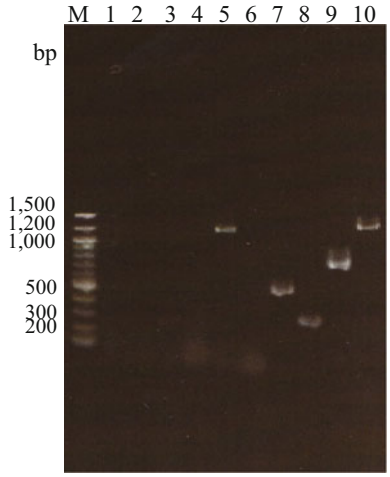


Fig. 5 RT-PCR analyses of the p-*Cas*, *Cas1* and *Cas2* gene transcripts expressed in the UPTC CF89-12 cells. *Lane M*, 100 bp DNA ladder (New England BioLabs Inc.). *Lanes 1–6*, with the UPTC CF89-12 total cellular RNA and without the UPTC CF89-12 genome DNA; *lanes 7–10*, without the RNA and with the DNA; *lanes 1*, *6*, without the reverse transcriptase (negative-control); *lanes 2–5*, with the reverse transcriptase (Sensiscript and Omniscript) and HotSarTaq DNA polymerase; *lanes 7–10*, with HotStar Taq DNA polymerase. Three primer pairs (f-/r-*Cas1*UPTC, *lanes 1*, *2*, *7*; f-/r-*Cas2*UPTC, *lanes 3*, *8*; f-/r-p-*Cas*UPTC, *lanes 4* and *9*) were employed in the present RT-PCRs, respectively. In addition, another PCR primer pair (f-/r-MOMP-common) was employed for the total cellular RNA positive-control of the UPTC CF89-12 (*lanes 5*, *6*, *10*)

positive-control for the total cellular RNA, was shown to occur (lane 5 in Fig. 5). Thus, no in vivo transcription of the p-*Cas*, *Cas1* and *Cas2* genes was confirmed in the UPTC CF89-12 cells (lanes 2, 3 and 4 in Fig. 5). In addition, three positive signals for corresponding gene segments occurred with UPTC CF89-12 DNA and without the RNA, as shown in lanes 7, 8 and 9 in Fig. 5.

Discussion

Campylobacter lari RM2100, whose genome analysis has already been described (Miller et al. 2008), was shown not to carry any CRISPRs or *Cas*, as described above. However, in the present study, we firstly identified the CRISPR locus (7, 500 bp) in a representative *C. lari* taxon, namely UPTC, in the Japanese UPTC CF89-12 isolate.

In Fig. 6, schematic representations of the CRISPRs and *Cas* loci in the five *C. jejuni* isolates, whose genome sequences have already been completed (accessible in the DDBJ/EMBL/GenBank databases) are illustrated for comparison.

Figures 1 and 6 also indicate that UPTC CF89-12 and five *C. jejuni* isolates contained p-*Cas* and two *Cas* genes, *Cas1* and *Cas2* within the CRISPR and *Cas* loci, similarly. Regarding the CRISPRs consensus sequence repeats, four

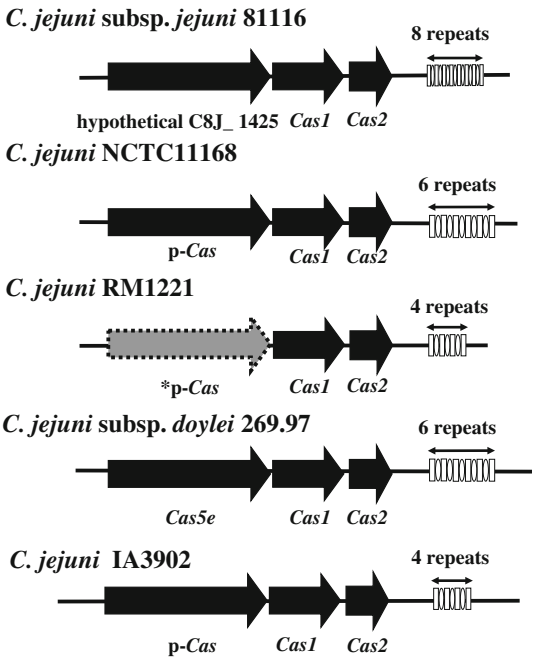


Fig. 6 Schematic representations of the CRISPR and *Cas* loci in the five *C. jejuni* isolates whose genome sequences have already been completed and are accessible in DDBJ/EMBL/GenBank. *Author's annotated (*C. jejuni* RM1221)

to 12 CRISPRs consensus sequence repeats separated by non-repetitive unique spacer regions (Fig. 6) occurred in these six UPTC and *C. jejuni* isolates. Nucleotide sequence alignment analysis of these CRISPRs consensus sequence repeats was carried out among the six *Campylobacter* isolates, as shown in Fig. 7. Surprisingly, the nucleotide sequence similarity of these CRISPRs consensus sequence repeats were shown to be approximately 92–100% among the UPTC CF89-12, *C. jejuni* 81116, NCTC11168, RM1221 subsp. *doylei* 269.97 and IA3902, and the sequence differences occurred within the three bases among these six isolates. However, no sequence similarity occurred among the unique spacer regions within the CRISPRs and *Cas* loci from these isolates (Fig. 2).

In addition, Bolotin et al. (2005) suggested that the unique spacer regions are the traces of past invasions by extrachromosomal elements. Therefore, it would be worthwhile to clarify if the CRISPRs spacer regions (29–31 bp) which occurred in the UPTC isolate CF89-12, are homologous to other extrachromosomal genes or not. Then, we compared the nucleotide sequences of the 11 CRISPRs unique spacer regions with the other sequences already reported. Consequently, the non-repetitive unique spacer regions nos. 7 and 9 in Fig. 2 were 100% identical to the sequences in the pCL2100 megaplasmid genome DNA (46,201 bp) and *C. lari* RM2100 genome DNA (1,525,460 bp) (Miller et al. 2008), respectively. Regarding the no. 9, the nucleotide sequence was also identified to be

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UPTC  CF89-12                  1 ATTTTATCATAAAGAAATTTAAAAAGAGACTAAAAC  36
C.jejuni  81116                1 ......C.................G.........  36
C.jejuni  NCTC11168            1 ......C.................G.........  36
C.jejuni  RM1221               1 ......C.................G........-  35
C.jejuni  subsp.doylei  269.97 1 ......C.................G........-  35
C.jejuni  IA3902               1 ......C.................G.........  36
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Fig. 7 Nucleotide sequence alignment analysis of the CRISPRS consensus sequence repeats among one UPTC and five *C. jejuni* isolates. *Numbers* at the *left* and *right* refer to the nucleotide positions of each CRISPRs consensus sequence repeats among six isolates. *Dots* indicate identical bases; changes are indicated so; *dashes* are deletions; identical positions in all cases are marked with *asterisks*

Table 1 Nucleotide sequence similarity of C*as1* (upper right) and C*as2* (lower left) among five *C. jejuni* and one UPTC CF89-12 isolates

	Campylobacter	1	2	3	4	5	6
1	*C. jejuni* 81116		97.2	97.5	97.4	97.2	70.8
2	*C. jejuni* NCTC11168	97.7		98.4	99.3	100.0	71.2
3	*C. jejuni* RM1221	99.5	97.7		98.4	98.4	70.8
4	*C. jejuni* subsp. *doylei* 269.97	97.9	99.8	97.5		99.3	70.9
5	*C. jejuni* IA3902	97.7	100.0	97.7	99.8		71.2
6	UPTC CF89-12	71.8	70.7	71.8	70.7	70.7	

Cas CRISPR associated, *UPTC* urease-positive thermophilic *Campylobacter*

Table 2 Amino acid sequence similarity of the putative ORFs for C*as1* (upper right) and *Cas2* (lower left) among five *C. jejuni* and one UPTC CF89-12 isolates

	Campylobacter	1	2	3	4	5	6
1	*C. jejuni* 81116		97.6	98.3	97.3	97.6	66.6
2	*C. jejuni* NCTC11168	99.3		98.6	98.6	100.0	67.9
3	*C. jejuni* RM1221	100.0	97.7		98.0	98.6	67.2
4	*C. jejuni* subsp. *doylei* 269.97	99.3	100.0	99.3		98.6	67.2
5	*C. jejuni* IA3902	99.3	100.0	99.3	100.0		67.9
6	UPTC CF89-12	65.4	66.4	65.4	66.4	66.4	

ORF open reading frame, *Cas* CRISPR associated, *UPTC* urease-positive thermophilic *Campylobacter*

100% identical to the sequence of the *C. lari* integrated element 1 (CLIE1, Cla_0845; np 803,155–803,183) in *C. lari* RM2100 (Miller et al. 2008). This may indicate that the no. 9 sequence can be derived from prophage following phage infection.

Nucleotide and amino acid sequence similarities of the *Cas1* and *Cas2* among the six isolates including UPTC CF89-12 are also shown in Tables 1 and 2. Nucleotide and amino acid sequence similarities of the *Cas1* and *Cas2* were very high (97.2–100%) among these five *C. jejuni* isolates, respectively. However, nucleotide and amino acid sequence similarities of the *Cas1* and *Cas2* were not so high i.e. 70.7–71.8 and 65.4–66.4% between UPTC CF89-12 and the five *C. jejuni* isolates, respectively.

The CRISPRs and *Cas* locus within the UPTC CF89-12 isolate was identified to exist between the TRMU and PGPase structural genes, both giving high sequence similarities to those two counterparts in the *C. lari* RM2100 strain, as described above. In addition, in the *C. lari* RM2100 strain, a hypothetically possible lipoprotein structural gene (423 bp) (140 aa residues for the ORF, reverse direction) occurred between these two counterparts. Therefore, in the UPTC CF89-12, the CRISPRs and *Cas* locus may be transfered and introduced into the genomic DNA from any extrachromosomal origin by a homologous recombination.

In the present study, no RT-PCR but positive PCR signals for the p-*Cas, Cas1* and *Cas2* genes were confirmed in the UPTC CF89-12 cells. Most recently, regarding these results, Pul et al. (2010) described that the DNA-binding protein H-NS suppresses CRISPR-*Cas* gene expression in *E. coli* K12 cells (Pul et al. 2010). Therefore, none of these

RT-PCR signals may possibly be caused due to the similar suppression of the CRISPR and *Cas* genes expression in the UPTC cells.

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