Genetic heterogeneity of the *dnaK* gene locus including transcription terminator region (TTR) in *Campylobacter lari*

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ABSTRACT

Nucleotide sequences of approximately 3.1 kbp consisting of the full-length open reading frame (ORF) for *grpE*, a non-coding (NC) region and a putative ORF for the full-length *dnaK* gene (1860 bp) were identified from a urease-positive thermophilic *Campylobacter* (UPTC) CF89-12 isolate. Then, following the construction of a new degenerate polymerase chain reaction (PCR) primer pair for amplification of the *dnaK* structural gene, including the transcription terminator region of *C. lari* isolates, the *dnaK* region was amplified successfully, TA-cloned and sequenced in nine *C. lari* isolates. The *dnaK* gene sequences commenced with an ATG and terminated with a TAA in all 10 isolates, including CF89-12. In addition, the putative ORFs for the *dnaK* gene locus from seven UPTC isolates consisted of 1860 bases, and the four urease-negative (UN) *C. lari* isolates included *C. lari* RM2100 reference strain 1866. Interestingly, different probable ribosome binding sites and hypothetically intrinsic ρ-independent terminator structures were identified between the seven UPTC and four UN *C. lari* isolates, respectively. Moreover, it is interesting to note that 20 out of a total of 28 polymorphic sites occurred among amino acid sequences of the *dnaK* ORF from 11 *C. lari* isolates, identified to be alternatively UPTC-specific or UN *C. lari*-specific. In the neighbour-joining tree based on the nucleotide sequence information of the *dnaK* gene, *C. lari* forms two major distinct clusters consisting of UPTC and UN *C. lari* isolates, respectively, with UN *C. lari* being more closely related to other thermophilic campylobacters than to UPTC.

KEY WORDS: Amino acid sequence.
Campylobacter lari.
dnaK.
Nucleotides.

Introduction

Thermophilic *Campylobacter* species, primarily *C. jejuni* and *C. coli* are the recognised cause of bacterial diarrhoea worldwide. *C. lari* is a thermophilic *Campylobacter* species that is resistant to nalidixic acid, one of the compounds generally used to discriminate this species from *C. jejuni* and *C. coli*.[1,2]

C. lari organisms were first isolated from mammalian and avian species, particularly seagulls of the genus *Larus*.[1,2] *C. lari* is also a cause of clinical infection.[3,4] An atypical group of isolates of urease-positive thermophilic campylobacters (UPTC) was first isolated from the natural environment in England in 1985.[5] Thereafter, this organism was described as a biovar or variant of *C. lari*.[6,7] Additional isolates of UPTC have also been reported in France,[6,8] Ireland,[9–11] The Netherlands[12] and Japan.[13,14] Thus, these two representative taxa, namely urease-negative (UN) *C. lari* and UPTC, occur within the species of *C. lari*.[15]

Heat shock proteins (HSPs) are generated in cells in response to a variety of physical and chemical stresses.[16] DnaK (HSP70) and GroEL (HSP60) are the most highly conserved proteins known, and are two major bacterial HSPs.[17–21] Descriptions of HSPs from thermophilic campylobacters have been published.[22–24] Genomic sequencing has revealed the presence of HSP homologues, also found in other thermophilic *Campylobacter* strains.[25,26]

The authors have reported a study on the biochemical isolation and identification of DnaK and GroEL from UPTC;[27] however, there have been no reports to date of the analysis of the *dnaK* gene sequence from thermophilic *C. lari*, consisting of UN *C. lari* and UPTC. Therefore, the present study aims to clone, sequence and characterise the full-length *dnaK* gene and its adjacent genetic loci from *C. lari* isolates and to compare the molecular characteristics with those of the other thermophilic campylobacters.

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Materials and methods

Isolates (n=10) of *C. lari* were used in the present study (Table 1). *C. lari* cells were cultured on blood agar base No. 2 (Oxoid, Hampshire, UK) containing defibrinated horse blood (7% [v/v]; Nippon Bio-test, Tokyo, Japan), supplemented with Butzler *Campylobacter*-selective medium (Nissui, Tokyo, Japan), under microaerophilic conditions at 37°C for 48 h. Genomic DNA was prepared from *C. lari* cells

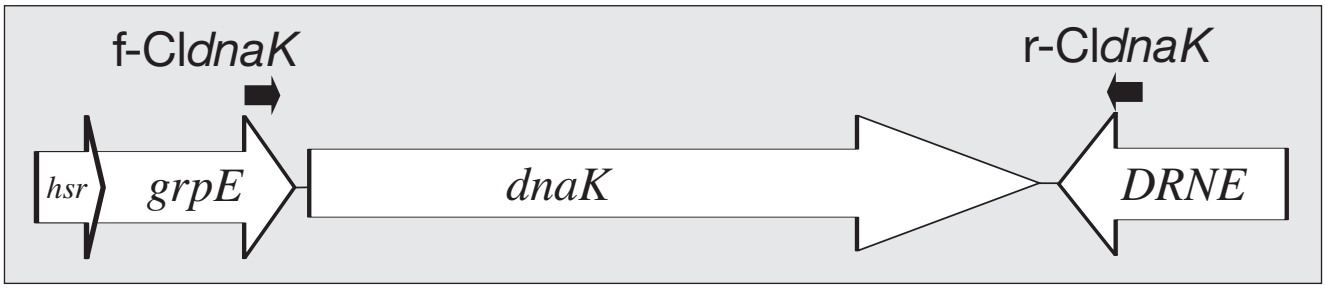

Fig. 1. A schematic representation of the *dnaK* gene and its adjacent genetic loci in UPTC CF89-12 (AB292221) determined in the present study, and *C. lari* RM2100 (AAFK00000000),[26] including the locations of a new primer pair for PCR amplification.

by sodium dodecyl sulphate (SDS) and proteinase K treatment, phenol-chloroform extraction and ethanol precipitation.[28]

For the construction of a size-selected partial library, genomic DNA from UPTC CF89-12 cells was partially digested with *Hin*dIII and 1.5–3.1 kbp fragments were prepared by 0.7% (w/v) agarose gel electrophoresis in 0.5 x TBE buffer. These fragments were then ligated into a *Hin*dIII-digested pUC19 vector after alkaline phosphatase treatment. The ligated recombinant DNA was transformed into competent *Escherichia coli* DH5α cells.[28]

The transformants were selected on LB agar containing ampicillin (50 μg/mL), X-gal (40 μg/mL) and IPTG (0.1 mmol/L). White colonies were subcultured on LB-ampicillin agar.

The library was screened with a probe specific for the *dnaK* structural gene of *C. jejuni* JCM2013, prepared using a degenerate oligonucleotide primer pair, 5′-GGWATWGAYYTDGGIACIACHAAYTC-3′ and 5′-CCWGCDATIYDCCIGCRTCYTT- 3′ (data not shown).[24]

Initially, six positive plasmid clones specific for the *dnaK* gene of the UPTC CF89-12 were obtained. Then, plasmid DNA was extracted by an alkaline-SDS purification procedure from these plasmid clones. All recombinant plasmid inserts were sequenced using the Texas red-labelled primer pair of M13.

Then, a degenerate PCR primer was designed comprising f-Cl*dnaK* (5′-GATCAGCMAAAGTTAGYGTTGC- 3′) and r-Cl*dnaK* (5′-ATGATGATAARCCWAAACGC- 3′) *in silico* for amplification of the full-length *dnaK* gene, including the terminator region (approximately 2 kbp) of *C. lari* isolates, based on the sequence information of the *dnaK* gene and the adjacent genetic loci of the UPTC CF89-12 isolate determined in the present study and *C. lari* RM2100 (AAFK00000000),[26] as shown in Figure 1.

The PCR mixture contained 1 μg template DNA, 10 mmol/L Tris-HCl (pH 8.3), 50 mmol/L KCl, 2 mmol/L $MgCl_2$, 400 μmol each dNTP, 1 μmol each primer, and a total of 1 unit of r*Taq* DNA polymerase (TaKaRa Bio Inc. Shiga, Japan). The PCR was performed in a 50 μL volume at 95°C for 3 min, followed by 30 cycles at 95°C for 1.5 min, 60°C for 1 min, 72°C for 3 min, and finally 72°C for 3 min.

Amplified PCR products were separated by 1% (w/v) agarose gel electrophoresis in 0.5 x TBE, then extracted and purified from the gel, as described previously.[29] Purified PCR products were cloned in a pGEM-T vector (Promega, Tokyo, Japan) using the TA cloning procedure.

Table 1. Isolates of *C. lari* and some thermophilic *Campylobacter* reference strains used in the present study, their putative ORF and CMW, and accession numbers of the nucleotide sequence data of the full-length *dnaK* gene accessible in DDBJ/EMBL/GenBank.

Campylobacter	Source	Country	ORF	Number of amino acids	CMW	Accession No.
UPTC CF89-12	River water	Japan	1860	620	67,040	AB292221
UPTC CF89-14	River water	Japan	1860	620	67,040	AB292222
UPTC NCTC12894	Sea water	England	1860	620	67,126	AB292223
UPTC NCTC12895	Mussel	England	1860	620	67,126	AB292224
UPTC 163	Mussel	N. Ireland	1860	620	67,078	AB292225
UPTC A3	Seagull	N. Ireland	1860	620	67,085	AB292226
UPTC 92251	Human	France	1860	620	67,054	AB292227
C. lari 48	Food	N. Ireland	1866	622	67,341	AB292228
C. lari 84C-1	Human	N. Ireland	1866	622	67,387	AB292229
C. lari JCM2530^T	Seagull	Japan	1866	622	67,341	AB292230
C. lari RM2100	Human	USA	1866	622	67,387	AAFK00000000
C. jejuni RM1221	Chicken	UK	1869	623	67,415	CP000025
C. jejuni NCTC11168	Human	USA	1869	623	67,417	AL111168
C. coli RM2228	Chicken	USA	1869	623	67,415	AAFL00000000
C. upsaliensis RM3195	Human	USA	1860	620	67,406	AAFJ00000000

UPTC: urease-positive thermophilic *Campylobacter*; ORF: open reading frame; CMW: calculated molecular weight.

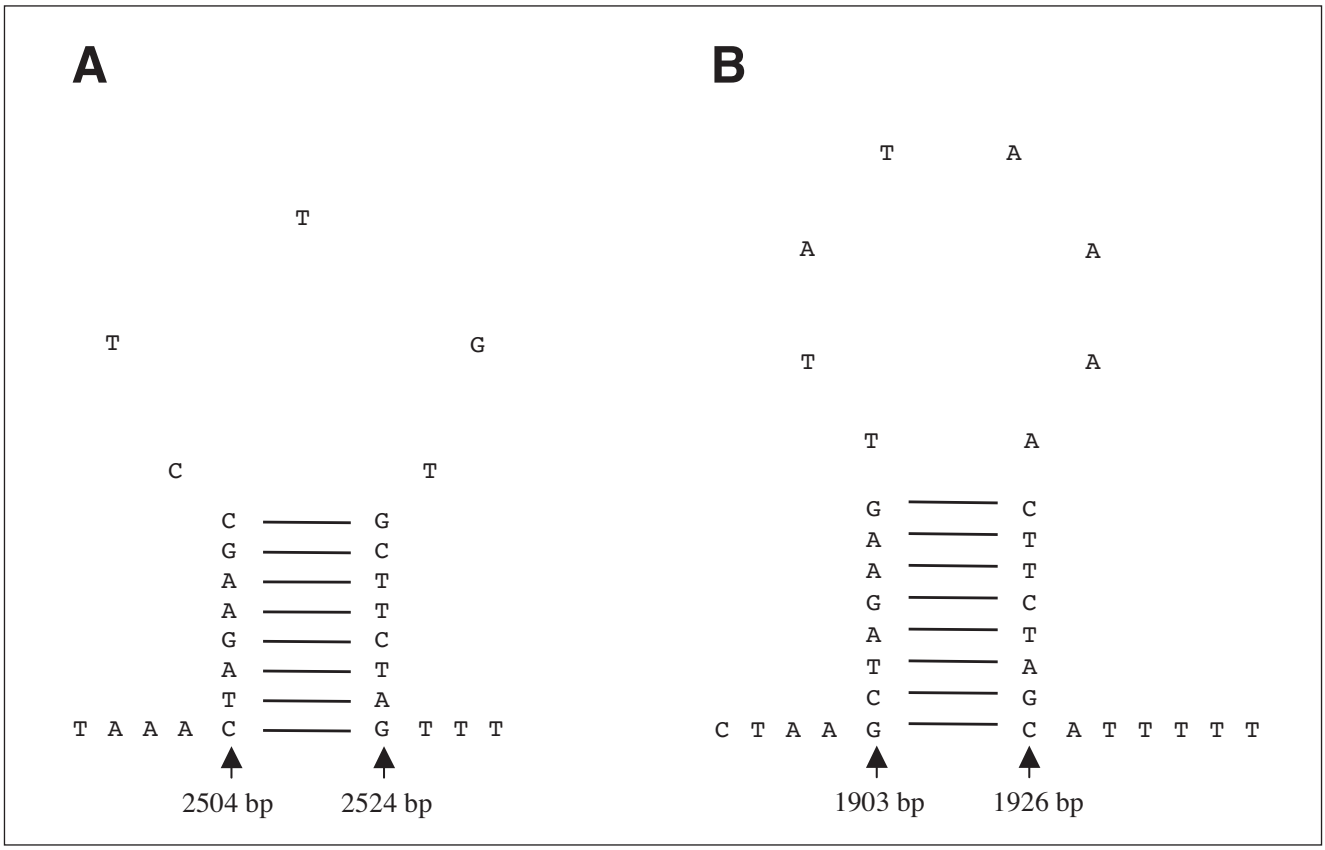

Fig. 2. Two hypothetically intrinsic ρ-independent transcription terminator structures that contain G+C rich region near the base of the stem, and a single-stranded run of T residues for the UPTC (A) and UN *C. lari* (B) isolates, respectively. Nucleotide positions (bp) are prepresented with UPTC CF89-12 (A) and *C. lari* JCM2530^T (B), respectively.

Following the nucleotide sequencing reaction with M13, sequencing of the amplicons was performed using an Hitachi SQ5500-EL DNA autosequencer. Sequence analysis was carried out using the Genetyx-Mac (version 9; Genetyx, Tokyo, Japan) computer software. At present, for accurate sequencing, multiple TA-cloned PCR products were sequenced. However, the sequences corresponding to the PCR primer of f-Cl*dnaK* and r-Cl*dnaK* were excluded from the sequence data of the approximately 2 kbp region containing the full-length *dnaK* gene accessible in DDBJ/EMBL/GenBank and further similarity analysis.

Nucleotide sequences of approximately 2 kbp of the full-length *dnaK* structural gene from the *C. lari* isolates were compared to each other and with the accessible sequence data from other thermophilic campylobacters, respectively, by employing CLUSTAL W software (1.7 program)[30] which was incorporated in the DDBJ. Following this, a phylogenetic tree was constructed by the neighbour-joining (NJ) method.[31]

Results

In this cloning and sequencing study, the sequences encoding a partial and putative open reading frame (ORF: 101 bp), a putative and full-length *grpE* ORF (507 bp), a 22 bp non-coding (NC) region, a putative and full-length *dnaK* ORF of 1.9 kbp, an NC terminator and a partial and putative ORF (571 bp) were identified in a partially digested fragment of the genomic DNA with *Hin*dIII from the Japanese UPTC CF89-12 isolate. The present sequence analysis clearly identified two partial and putative ORFs (33 and 190 amino acid residues) from the UPTC isolate, which has high amino acid sequence similarities to the heat shock regulator (*hsr*; approximately 91%) from the *C. lari* RM2100 (EAL55461), upstream of the 22-bp NC region, and to the DNA RNA non-specific endonuclease (*DRNE*; 93%), and downstream of the hypothetically intrinsic ρ-independent transcription terminator, respectively.

The putative ORF for the *dnaK* included the 1860 base nucleotide sequence (nucleotide position 633–2492 bp for the UPTC CF89-12; AB292221) and was predicted to encode peptides of 620 amino acid residues with the calculated molecular weight (CMW) of 67,040 (Table 1). In the present study, the nucleotide positions used are those of the UPTC CF89-12 isolate (AB292221). The putative ORF commenced with an ATG start codon and terminated with a TAA stop codon. and its G+C content was approximately 33.4%.

A probable ribosome-binding (RB) site (Shine-Dalgarno [SD] sequence)[32] complementary to a highly conserved sequence of CCUCCU close to the 3′ end of 16S ribosomal RNA (AGGA [623–626 bp]) was identified for the *dnaK* gene.

The full-length *dnaK* gene from the UPTC CF89-12 isolate showed a nucleotide sequence similarity of 91.0% to *C. lari* RM2100, 86.0% to *C. jejuni* RM1221[26] and NCTC11168[25] strains (Table 2). Regarding the hypothetically intrinsic ρ-independent transcription termination region, quite similar terminator structures were identified to that of UPTC CF89-12 (Fig. 2A) in the other six UPTC isolates. However, in the three UN *C. lari* isolates, different nucleotide sequences were identified in these regions and different hypothetically

Table 2. Sequence similarities (%) of the nucleotide (upper right) and amino acid (lower left) of the putative ORFs of the *dnaK* gene from the *C. lari* isolates and other thermophilic campylobacters used as references.

Campylobacter	UPTC CF89-12	UPTC CF89-14	UPTC NCTC12984	UPTC NCTC12985	UPTC 163	UPTC A3	UPTC 92251
UPTC CF89-12		100	97.42	97.21	96.40	97.05	97.10
UPTC CF89-14	100		97.42	97.21	96.40	97.05	97.10
UPTC NCTC12984	99.68	99.68		99.25	96.46	97.37	97.53
UPTC NCTC12985	99.68	99.68	100		96.40	97.26	97.21
UPTC 163	99.36	99.36	99.36	99.36		96.35	96.03
UPTC A3	99.52	99.52	99.52	99.52	99.52		98.34
UPTC 92251	99.52	99.52	99.52	99.52	99.52	99.68	
C. lari 48	96.47	96.47	96.47	96.47	96.15	96.47	96.31
C. lari 84C-1	96.15	96.15	96.15	96.15	95.83	96.15	95.99
C. lari JCM2530	96.47	96.47	96.47	96.47	96.15	96.47	96.31
C. lari RM2100	96.15	96.15	96.15	96.15	95.83	96.15	95.99
C. jejuni RM1221	93.74	93.74	93.90	93.90	93.59	93.74	93.58
C. jejuni NCTN11168	93.74	93.74	93.90	93.90	93.59	93.74	93.58
C. coli RM2228	93.74	93.74	94.38	94.38	94.23	94.22	94.06
C. upsaliensis 3195	94.06	94.06	92.94	92.94	92.59	92.94	92.78

intrinsic ρ-independent transcription terminator structures were identified from the UPTC isolates (Fig. 2B).

The putative ORF for the full-length *grpE* gene (encoding a heat shock protein in *E. coli* essential for phage lambda growth at all temperatures and for the host cell growth above 43°C)[33] from the Japanese UPTC CF89-12 isolate included the 507 base nucleotide sequence (101–607 bp) and was predicted to encode peptides of 169 amino acid residues with a CMW of 19,389. The putative ORF commenced with a GTG start codon and terminated with a TAA. The RB site (AGGA; 91–94 bp) was also identified for the *grpE* gene.

A possible overlap was identified among eight nucleotides, including the four between the GTGA (101–104 bp; TGA, the most probable stop codon) for the *hsr* gene and the GTGA (GTG, the most probable start codon) for the *grpE* gene within the UPTC CF89-12, as well as *C. lari* RM2100 (AAFK00000000). The full-length *grpE* gene from the UPTC CF89-12 isolate showed nucleotide sequence similarities of 92.6% to *C. lari* RM2100 and 71.5–71.6% to *C. jejuni* RM1221 and NCTC11168 strains, respectively (Table 3).

Following the construction of a new degenerate PCR primer pair (f-C1*dnaK*/r–C1*dnaK*) *in silico* for amplification of the full-length *dnaK* gene, including the transcription terminator region of the *C. lari* isolates, the authors successfully amplified, TA-cloned and sequenced those (approximately 2050 bp) from the additional nine *C. lari* isolates (UN C. lari [n=3], UPTC [n=6]). This primer pair was constructed for amplification of the region, including the NC region upstream of the *dnaK*, the full-length *dnaK* structural gene and the NC terminator structure from the *C. lari* isolates. At present, in all nine isolates of *C. lari*, these regions were identified to occur following TA-cloning, sequencing and sequence analysis.

Regarding the full-length *dnaK* structural gene, all the genes commenced with an ATG start codon and terminated with a TAA stop codon in all nine isolates. In addition, as shown in Table 1, the putative ORFs of the *dnaK* gene from the seven isolates of UPTC, including UPTC CF89-12 described above, comprised 1860 bases and 620 amino acid residues, and the four UN *C. lari* isolates, including the *C. lari* RM2100[26] reference strain, comprised 1866 bases and 622 amino acid residues. These also differed from those of the other thermophilic campylobacters (1869 bases and 623 amino acid residues for *C. jejuni* RM1221 and NCTC11168 and *C. coli* RM2228; 1860 bases and 620 amino acid residues for *C. upsaliensis* RM3195). Interestingly, at RB sites, AGGA

Table 3. Sequence similarities (%) of the nucleotide (upper right) and amino acid (lower left) of the putative ORFs of the *grpE* from the UPTC CF89-12 isolate and other thermophilic campylobacters used as references.

Campylobacter	UPTC CF89-12	*C. lari* RM2100	*C. jejuni* RM1221	*C. jejuni* NCTC11168	*C. coli* RM2228	*C. upsaliensis* RM3195
UPTC CF89-12		92.55	71.46	71.62	71.99	75.15
C .lari RM2100	95.29		71.83	72.71	72.34	73.97
C. jejuni RM1221	69.83	69.83		93.79	93.60	72.78
C. jejuni NCTC11168	68.54	69.10	92.05		97.93	73.12
C. coli RM2228	69.83	69.27	92.09	98.31		72.74
C. upsaliensis RM3195	71.18	70.00	69.10	70.06	69.93	

C. lari 48	*C. lari* 84C-1	*C. lari* JCM2530	*C. lari* RM2100	*C. jejuni* RM1221	*C. jejuni* NCTC11168	*C. coli* RM2228	*C. upsaliensis* 3195
91.44	91.12	91.44	91.01	86.00	85.95	86.70	82.93
91.44	91.12	91.44	91.01	86.00	85.95	86.70	82.93
91.39	90.85	91.39	90.74	86.22	86.32	87.07	83.31
91.23	90.74	91.23	90.64	85.82	86.00	86.91	83.25
91.01	90.64	91.01	90.53	86.22	95.90	86.97	82.88
90.96	90.58	90.96	90.58	86.38	86.32	87.13	83.04
91.23	90.90	91.23	90.80	86.38	86.27	86.91	82.77
	96.90	100	96.90	85.47	85.42	86.22	82.45
99.68		96.90	99.79	85.47	85.26	85.84	82.13
100	99.68		96.90	85.47	85.42	86.22	82.45
99.68	100	99.68		85.36	85.15	85.95	82.08
94.22	93.90	94.22	93.90		99.15	93.06	84.51
94.22	93.90	94.22	93.90	99.84		93.27	84.57
94.54	94.22	94.54	94.22	98.07	98.23		83.82
93.42	93.42	93.42	93.42	95.99	95.83	95.35	

were identified for the *dnaK* gene among all the UPTC isolates and GAGGA among the UN *C. lari* isolates.

Levels of similarity of the aligned nucleotide sequences of the full-length *dnaK* structural genes from the 10 *C. lari* isolates (UPTC [*n*=7], UN *C. lari* [*n*=3]) examined in the present study are shown on the upper right in Table 2. The nucleotide sequences of the gene from the 11 *C. lari* isolates, including the RM2100 , showed 90.6–100% similarity to each other.

The *dnaK* genes were identical between the two Japanese UPTC isolates, CF89-12 and CF89-14, and between *C. lari* JCM2530^T and *C. lari* 48, respectively (Table 2). Moreover, the nucleotide sequences of the genes from the 11 *C. lari* isolates showed 85.2–87.1% similarity to those of *C. jejuni* RM1221, NCTC11168 and *C. coli* RM2228 strains (Table 2).

When the deduced amino acid sequence alignment analysis of the putative ORFs for the *dnaK* from the *C. lari* isolates, including the *C. lari* RM2100, was performed the 11 ORFs showed 96–100% similarity to each other, and 93.9–94.2% similarity to those of the *C. jejuni* RM1221, NCTC11168 and *C. coli* RM2228 reference strains (Table 2).

Discussion

In the present study, the full-length *dnaK* gene loci from *C. lari* isolates were examined using recombinant genomic DNA cloning and TA cloning following PCR amplification procedures. It is interesting to note that 20 out of 28 polymorphic sites occurring among amino acid sequences of the putative ORF of the full-length *dnaK* from the 11 *C. lari* isolates (UPTC [*n*=7 , UN *C. lari* [*n*=4]), including the *C. lari* RM2100 reference strain, are identified to be alternatively UPTC-specific or UN *C. lari*-specific, although the number of *C. lari* isolates examined are limited. In addition, the ORF of the full-length *dnaK* comprised 620 amino acid residues for UPTC and 622 amino acid residues for UN *C. lari*. The insertion of the two amino acid sequences (K and P) occurred at the amino acid positions of *C. lari* JCM2530^T (604 and 605 residues) in the UN *C. lari* isolates.

Regarding the hypothetically intrinsic ρ-independent transcription terminator structures, the UPTC-specific or UN *C. lari*-specific terminator structures also occurred for the *dnaK* gene, as shown in Figure 2.

In a previous report on the biochemical isolation and identification of DnaK and GroEL from UPTC,[27] the authors identified a 70 kDa protein of DnaK by SDS-PAGE and its 11 N-terminal amino acid sequence by an N-terminal protein sequencing method, for the Japanese UPTC CF89-12 isolate. The present cloning and nucleotide sequencing analysis of the *C. lari* isolates confirms the total amino acid sequences and the CMW (67,040) of the putative ORF for the full-length *dnaK* gene from the UPTC CF89-12 isolate.

A putative and partial ORF showing high sequence similarity with *DRNE* in all 10 *C. lari* isolates was identified in the present study, being transcribed and translated in the reverse direction to the other ORFs determined. Based on nucleotide sequence alignment analysis, the ORF may use a similar region of the intrinsic ρ-independent transcription terminator for the *dnaK* gene as its terminator in all 10 isolates. Thus, the UPTC-specific or UN *C. lari*-specific terminator structures may also occur for the putative and partial ORF for the *DRNE* gene.

Previously, the authors reported four nucleotide sequence differences found in the *flaA*-like sequences of about 1.5 kbp in length between the Japanese UPTC CF89-12 and CF89-14 isolates, which suggested two distinctly different isolates.[29,34] However, these two isolates were shown to carry an identical nucleotide sequence of approximately 2 kbp, comprising a 22 bp NC region, the full-length *dnaK* structural gene (1863 bp) and its terminator. This indicates that these two isolates have a close genetic relationship.

E. coli dnaK is composed of three domains, an N-terminal ATPase, a substrate peptide-binding and a C-terminal α-helical domain.[35] Interestingly, aligned amino acid sequences of the putative *dnaK* ORF from the 11 *C. lari*

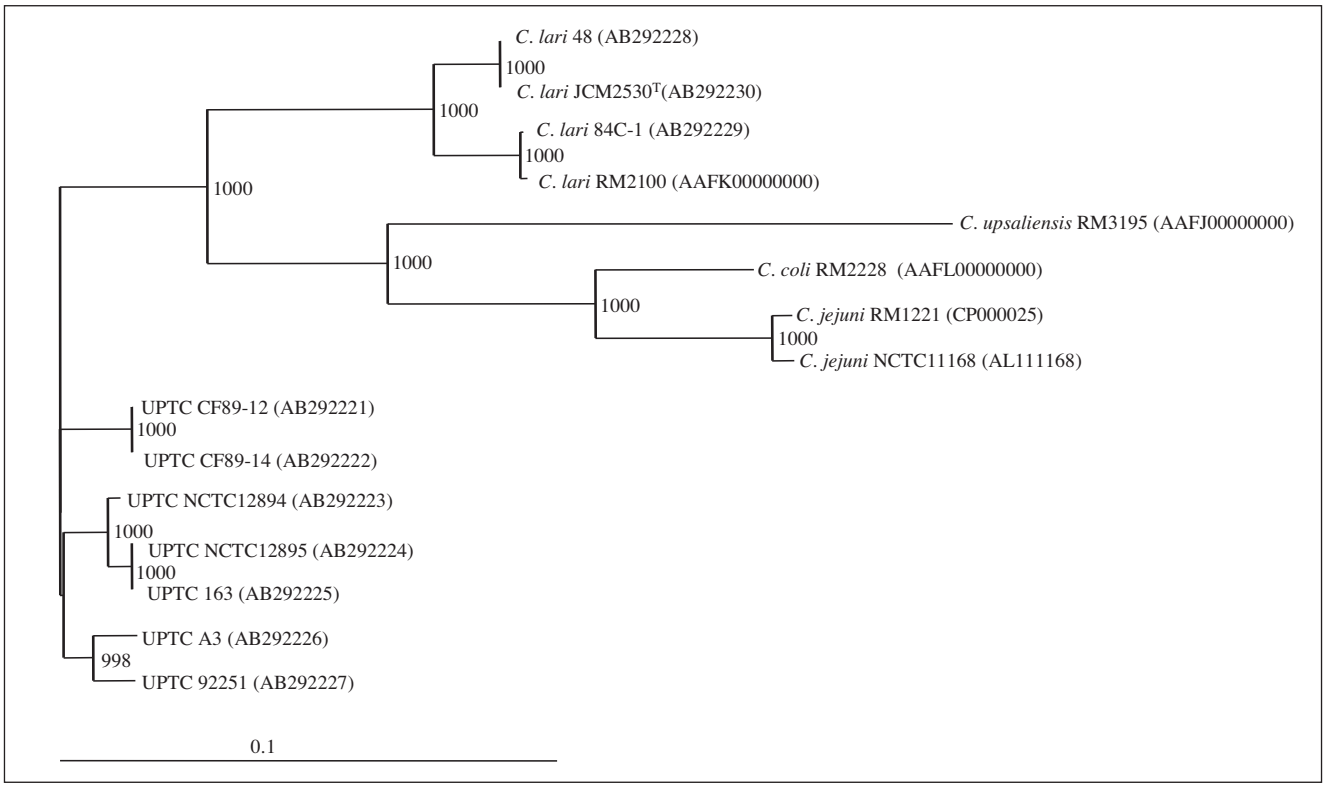

Fig. 3. A phylogenetic tree based on the sequence similarity data of the *dnaK* structural gene from *C. lari* isolates and other thermophilic campylobacters. The tree was constructed by the NJ method. Boot-strap values of 1000 are shown at the branch point. Bar, 0.1 (evolutionary distance). The accession numbers for the sequences used in the present study are shown in parentheses.

isolates examined showed that the substrate peptide-binding domain is most highly conserved, compared with the two other domains (data not shown).

Figure 3 is a dendrogram showing phylogenetic relationships constructed by the NJ method,[31] based on the nucleotide sequence information from the full-length *dnaK* structural gene from the 11 *C. lari* isolates, including the RM2100 reference strain. The dendrogram forms two distinct major clusters consisting of the UPTC and UN *C. lari* isolates, including the other three thermophilic campylobacters, *C. jejuni, C. coli* and *C. upsaliensis*. Interestingly, based on sequence data of the *dnaK* gene locus, UN *C. lari* is more closely related to the other thermophilic *Campylobacter* species than it is to UPTC. Thus, the nucleotide sequence information of the full-length *dnaK* gene may discriminate the two *C. lari* taxa, UPTC and UN *C. lari*, although the number of isolates examined in the present study is small. □

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References

1 Skirrow MB, Benjamin J. '1001' campylobacters: cultural characteristics of intestinal campylobacters from man and animals. *J Hyg (Camb)* 1980; **85**: 427–42.

2 Benjamin J, Leaper S, Owen RJ, Skirrow MB. Description of *Campylobacter laridis*, a new species comprising the nalidixic acid-resistant thermophilic *Campylobacter* (NARTC) group. *Curr Microbiol* 1983; **8**: 231–8.

3 Nachamkin I, Stowell C, Skalina D *et al*. *Campylobacter laridis* causing bacteremia in an immunosuppressed patient. *Ann Int Med* 1984; **101**: 55–7.

4 Werno AM, Klena JD, Shaw GM, Murdoch DR. Fatal case of *Campylobacter lari* prosthetic joint infection and bacteremia in an immunocompetent patient. *J Clin Microbiol* 2002; **40**: 1053–5.

5 Bolton FJ, Holt AV, Hutchinson DN. Urease-positive thermophilic campylobacters. *Lancet* 1985; **i**: 1217–8.

6 Megraud F, Chevrierr D, Desplaces N, Sedallian A, Guesdon JL. Urease-positive thermophilic *Campylobacter* (*Campylobacter laridis* variant) isolated from an appendix and from human feces. *J Clin Microbiol* 1988; **26**: 1050–1.

7 Owen RJ, Costas M, Sloss L, Bolton FJ. Numerical analysis of electrophoretic protein patterns of *Campylobacter laridis* and allied thermophilic campylobacters from the natural environment. *J Appl Bacteriol* 1988; **65**: 69–78.

8 Bezian MC, Ribou G, Barberis-Giletti C, Megraud F. Isolation of a urease-positive thermophilic variant of *Campylobacter lari* from a patient with urinary tract infection. *Eur J Clin Microbiol Infect Dis* 1990; **9**: 895–7.

9 Wilson IG, Moore JE. Presence of *Salmonella* spp. and *Campylobacter* spp. in shellfish. *Epidemiol Infect* 1996; **116**: 147–53.

10 Kaneko A, Matsuda M, Miyajima M, Moore JE, Murphy PG. Urease-positive thermophilic strains of *Campylobacter* isolated from seagulls (*Larus* spp.). *Lett Appl Microbiol* 1999; **29**: 7–9.

11 Matsuda M, Kaneko A, Stanley T *et al*. Characterization of urease-positive thermophilic *Campylobacter* subspecies by multilocus enzyme electrophoresis typing. *Appl Environ Microbiol* 2003; **69**: 3308–10.

12 Endtz HP, Vliegenthart JS, Vandamme P *et al.* Genotypic diversity of *Campylobacter lari* isolated from mussels and oysters in the Netherlands. *Int J Food Microbiol* 1997; **34**: 79–88.
13 Matsuda M, Kaneko A, Fukuyama M *et al*. First finding of urease-positive thermophilic strains of *Campylobacter* in river water in the Far East, namely in Japan, and their phenotypic and genotypic characterization. *J Appl Bacteriol* 1996; **81**: 608–12.
14 Matsuda M, Shibuya T, Itoh Y *et al*. First isolation of urease-positive thermophilic *Campylobacter* (UPTC) from crows (*Corvus levaillantii*) in Japan. *Int J Hyg Environ Health* 2002; **205**: 321–4.
15 Matsuda M, Moore JE. Urease-positive thermophilic *Campylobacter* species. *Appl Environ Microbiol* 2004; **70**: 4415–8.
16 Lindquist S, Craig EA. The heat-shock proteins. *Annu Rev Genet* 1988; **22**: 631–77.
17 Zeilstra-Ryalls J, Fayet O, Georgopoulos C. The universally conserved GroE (Hsp60) chaperonins. *Annu Rev Microbiol* 1991; **45**: 301–25.
18 Gupta RS, Golding GB. Evolution of HSP70 gene and its implications regarding relationships between Archaebacteria, Eubacteria, and Eukaryotes. *J Mol Evol* 1993; **37**: 573–82.
19 Boorstein WR, Ziegelhoffer T, Craig EA. Molecular evolution of the HSP70 multigene family. *J Mol Evol* 1994; **38**: 1–17.
20 Gupta RS. Phylogenetic analysis of the 90 kD heat shock family of protein sequences and an examination of the relationship among animals, plants and fungi species. *Mol Biol Evol* 1995; **12**: 1063–73.
21 Segal R, Ron EZ. Regulation and organization of the groE and dnaK operons in Eubacteria. *FEMS Microbiol Lett* 1996; **138**: 1–10.
22 Takata T, Wai SN, Takade A *et al*. The purification of a GroEL-like stress protein from aerobically adapted *Campylobacter jejuni. Microbiol Immunol* 1995; *39*: 639–45.
23 Fujimoto S, Fujita M. Serum antibody level against GroEL type heat-shock protein of *Campylobacter jejuni* in patients with Guillain-Barre syndrome. *Microbiol Immunol* 1999; **43**: 241–4.
24 Thies FL, Karch H, Hartung HP, Giegerich G. Cloning and expression of the *dnaK* gene of *Campylobacter jejuni* and antigenicity of heat shock protein 70. *Infect Immun* 1999; **67**: 1194–200.
25 Parkhill J, Wren BW, Mungall K *et al*. The genome sequence of the food-borne pathogen *Campylobacter jejuni* reveals hypervariable sequences. *Nature* 2000; **403**: 665–8.
26 Fouts DE, Mongodin EF, Mandrell RE *et al*. Major structural differences and novel potential virulence mechanisms from the genomes of multiple *Campylobacter* species. *PLoS Biol* 2005; **3**: e15.
27 Murayama O, Tsuboi Y, Samata T, Moore JE, Matsuda M. Biochemical isolation and identification of DnaK and GroEL from urease-positive thermophilic campylobacters. *Br J Biomed Sci* 2003; **60**: 26–7.
28 Sambrook J, Russell DW. *Molecular cloning; a laboratory manual* 3rd edn. Cold Spring Harbor, New York: Cold Spring Harbor Laboratory Press, 2001.
29 Sekizuka T, Gondo T, Murayama O *et al*. flaA-like sequences containing internal termination codons (TAG) in urease-positive thermophilic *Campylobacter* isolated in Japan. *Lett Appl Microbiol* 2002; **35**: 185–9.
30 Thompson JD, Higgins DG, Gibson TJ. CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. *Nucleic Acids Res* 1994; **22**: 4673–80.
31 Saitou N, Nei M. The neighbor-joining method: a new method for reconstructing phylogenetic trees. *Mol Biol Evol* 1987; **4**: 406–25.
32 Benjamin L. *Genes VII.* Oxford: Oxford University Press, 2000.
33 Ang D, Georgopoulos C. The heat-shock-regulated *grpE* gene of *Escherichia coli* is required for bacterial growth at all temperatures but is dispensable in certain mutant backgrounds. *J Bacteriol* 1989; **171**: 2748–55.
34 Matsuda M, Sekizuka T, Murayama O, Moore JE. Two Japanese urease-positive thermophilic campylobacters, CF89-12 and CF89-14, isolated in 1989 prove to be two distinct strains. *Br J Biomed Sci* 2002; **59**: 65.
35 Suppini J-P, Amor M, Alix J-H, Ladjimi MM. Complementation of an *Escherichia coli* DnaK defect by Hsc70-DnaK chimeric proteins. *J Bacteriol* 2004; **186**: 6248–53.