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Cloning and sequence analysis of the *recA* gene in urease-positive thermophilic campylobacter (UPTC)

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The *recA* gene is essential for the homologous genetic recombination and for the post-replicative repair of DNA damage, and in responses induced by DNA-damaging agents.[1] Genetic analysis of *recA* in campylobacters has been performed,[2-4] but little work has been done on thermophilic campylobacters.[5]

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Fig. 1. *recA* gene fragments of UPTC amplified using a primer pair *recA* FL-f and *recA* FL-r. Lane KL, 1kb DNA ladder; lane L, 100 bp DNA ladder. Lane 1, UPTC NCTC 12894; lane 2, UPTC CF89-12; lane 3, UPTC A1; lane 4, *C. lari* JCM2530T; lane 5, *C. jejuni* 2013; lane 6, no template DNA (negative-control).

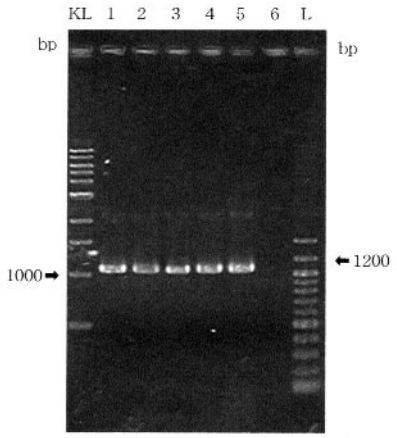


Table I. Origins of campylobacter isolates used in the present study

Isolate No.	Campylobacter	Source	Country
NCTC12894	UPTC	Sea water	England
CF89-I2[12]	UPTC	River water	Japan
AI[13]	UPTC	Seagull	N. Ireland
JCM2530[13]	*C. lari*	Seagull	Japan
JCM20I3	*C. jejuni*	Human	Japan

Urease-positive thermophilic campylobacter (UPTC), a microaerophilic and Gram-negative bacterium, is an organism only relatively recently identified in England.[6,7] After the original description, UPTC isolates were reported in France, Northern Ireland and The Netherlands, and, recently, strains were also found in Japan, where they were characterised both phenotypically and genotypically.[8-13] The aim of the present study is to clone and characterise the *recA* gene in UPTC and *Campylobacter lari*.

Strains of thermophilic campylobacters used in the present study are shown in Table 1. Genomic DNA for polymerase chain reaction (PCR) amplification was prepared by proteinase K treatment, phenol-chloroform extraction and ethanol precipitation.[14]

In the present study, a degenerate primer pair (*recA*FL-f and *recA*FL-r) used for PCR amplification of almost the full-length of the *recA* gene was designed from sequences of the gene in *C. jejuni* 81-176 (U03121)[5] and *C. fetus* 23D (AF020677),[15] taken from EMBL and GenBank. Primer sequences were as follows: *recA*FL-f 5′-GGAAA[A,C,G,T]T[A,C,G,T] ATGGATGATAAT-3′ and *recA*FL-r 5′-[A,C,G,T]A[A,C,G,T]CATTA[A,C,G,T]- TC[A,C, G,T]TCTCCTTC-3′.

PCR mixture contained 10 mmol/L Tris-HCl [pH 9.0], 50 mmol/L KCl, 1.5 mmol/L $MgCl_2$, 0.01% gelatine, 0.1% Triton

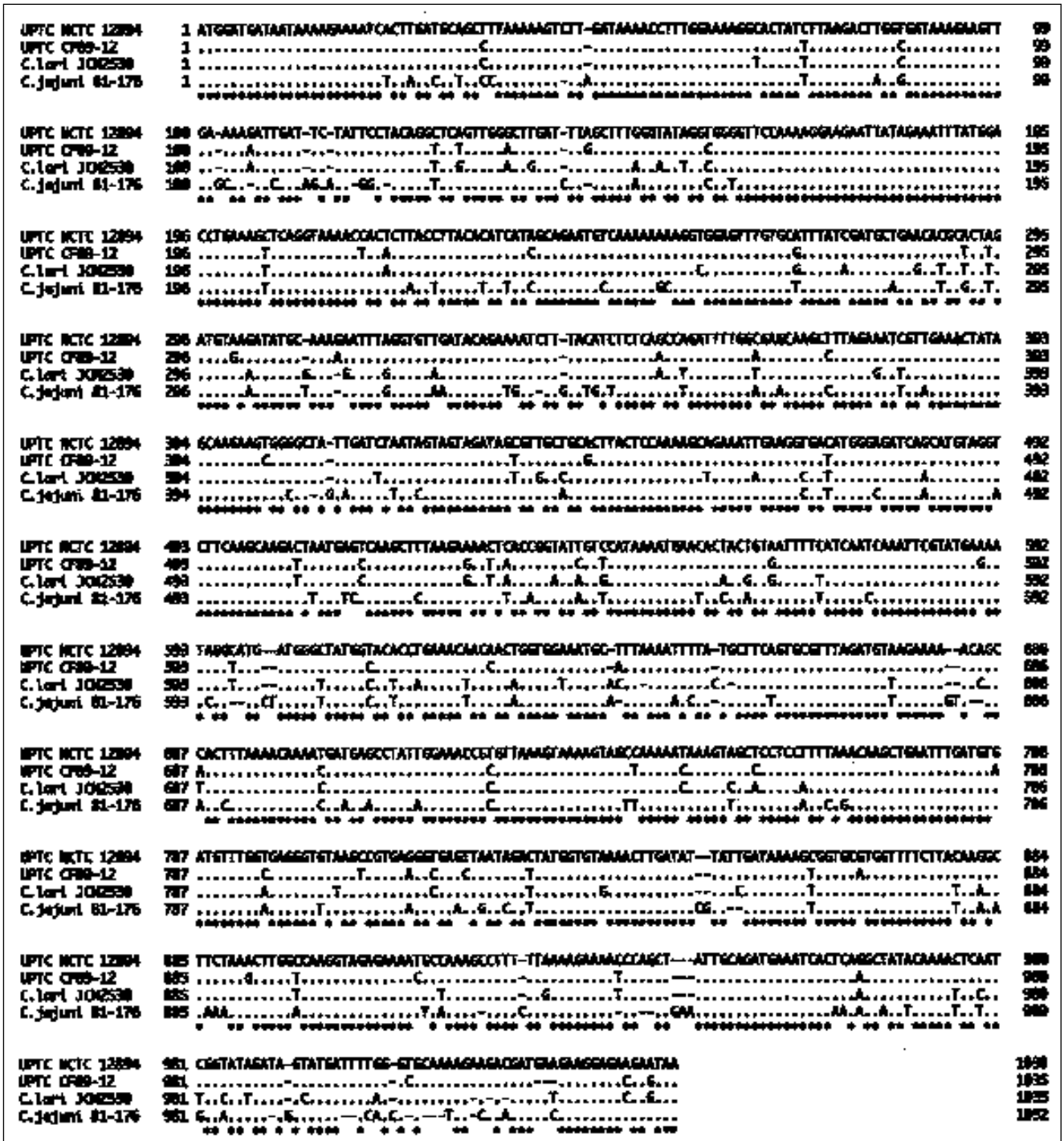


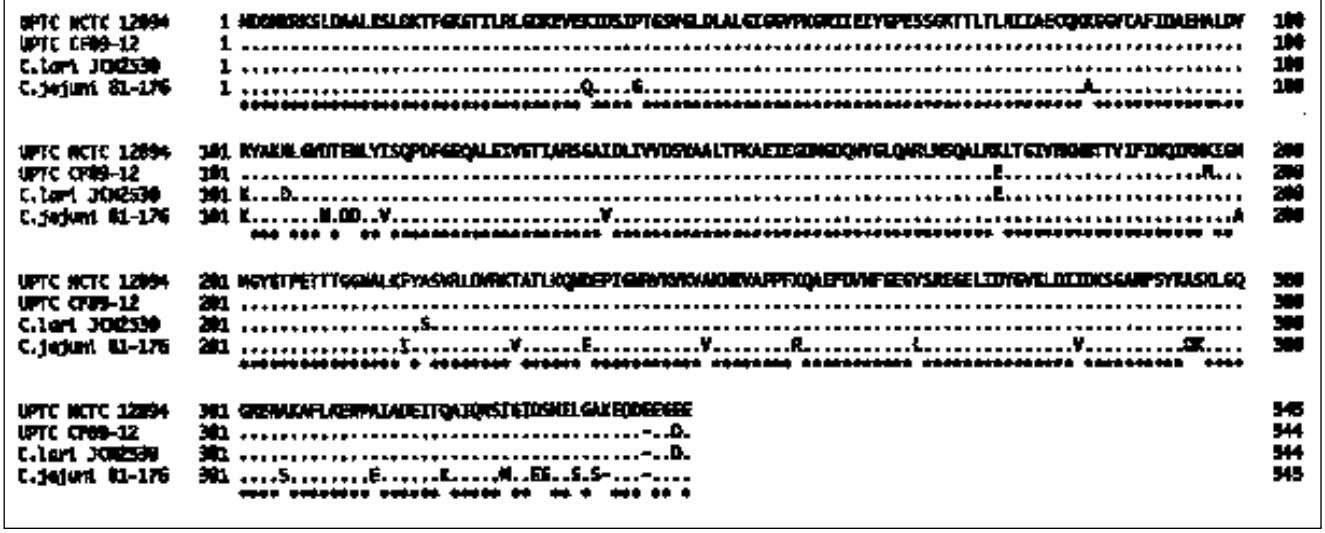
Fig.2. Nucleotide sequence alignment of the *recA* gene of *C. jejuni* 81-176[5] and almost the full-length segment (about 1000 bp in length) of the *recA* gene of strains of UPTC, NCTC12894, CF89-12 and *C. lari* JCM2530. Dots indicate identical base; changes are so indicated; dashes are deleted; positions identical in all strains are marked by asterisks. Numbers at the left and right refer to base pairs of almost the full-length segment of the *recA* gene amplified and sequenced in the present study.

X-100, 0.2 μmol/L each primer, 2.5 mmol/L each dNTPs, 300 ng template DNA and 2 units *Thermus aquaticus* (*Taq*) DNA polymerase. PCR was performed in a 50 μL volume for 3 min at 94˚C, followed by 40 cycles of 94˚C for 1 min, 42˚C for 1 min, 72˚C for 2 min, and finally 72˚C for 10 min. Amplified PCR products were electrophoresed in 1% (w/v) agarose gel.

For cloning of almost the full-length of the *recA* gene, PCR products were purified with a Geneclean II kit (Bio 101, Inc.) and inserted into a pGEM-T vector using the TA cloning procedure.[14] Following dideoxy sequencing reaction using a Thermo Sequenase pre-mixed cycle sequencing kit (Amersham Pharmacia Biotech), sequencing of the *recA* gene fragment was performed using an Hitachi SQ-5500L DNA autosequencer. Sequence analysis was performed using Genetyx-Mac (version 9.0) computer software.

recA gene sequences for *Escherichia coli* (EMBL/GenBank

Fig.3. Deduced amino acid sequence alignment of the possible ORF of the *recA* gene of strains of UPTC, NCTC12894, CF89-12 and *C. lari* JCM2530. Dots indicate identical residues; changes are so indicated; dashes are deleted; positions identical in all strains are marked by asterisks. Numbers at the left and right refer to amino acid residues of the possible ORF. Amino acids are designated by single-letter code.



accession number: X55552), *Erwinia carotovora* (X55554), *Frankia alni* (AJ006707), *Frankia* sp. (AJ006704), *Proteus vulgaris* (X55555), *Shigella flexneri* (X55553) and *Shigella sonnei* (AF101227) were taken from EMBL (http://.ebi.ac.uk) and GenBank (http://www.ncbi.nlm.nih.gov/Genbank). Nucleotide sequence data determined in the present study are accessible in the DDBJ (http://www.ddbj.nig.ac.jp)/ EMBL/GenBank, under accession numbers AB067767, AB067768 and AB074463.

PCR primer pair of *recA*FL-f and *recA*FL-r amplified almost the full length of the *recA* gene (about 1000 bp) of all three UPTC strains, a strain of *C. lari* and a strain of *C. jejuni* (Figure 1). Nucleotide sequencing after TA cloning of almost the full length (about 1000 bp) of the *recA* gene of two UPTC strains (NCTC12894, CF89-12) and *C. lari* (JCM2530) was carried out (Figure 2).

For UPTC NCTC12894, an open reading frame (ORF) of 1035 nucleotides encoded a predicted recA protein of 345 amino acids with a calculated Mr 37 314; figures for UPTC CF89-12 were 1032, 344 and 37 200; and for *C. lari* JCM2530 1032, 344 and 37 161 (Table 2). G+C content of approximately 36% within the putative ORF for the two UPTC strains, one *C. lari* strain and one *C. jejuni* strain, and approximately 39% for *C. fetus* (Table 2) were characteristic of the *Campylobacter* spp. genome.[16,17]

Nucleotide sequence comparison analysis of the possible ORF demonstrated that the two UPTC strains showed some 94% sequence homology, 90-92% sequence homology to *C. lari*, about 84% to *C. jejuni*, and 50-60% to other Enterobacteriaceae mentioned previously. Consequently, the primer pair used for PCR amplification of almost the full length of the *recA* gene used in the present study proved suitable for the genotypic analysis of the *recA* gene in UPTC and other thermophilic *Campylobacter* spp. In addition, the present study clearly suggests that nucleotide sequence differences of the *recA* gene may have discriminatory power among UPTC, *C. lari* and *C. jejuni*.

Alignment of the deduced amino acid sequence of the possible *recA* ORF for the two UPTC strains showed approximately 99% homology, with 98-99% homology to *C. lari* JCM2530 and 91% to *C. jejuni* 81-176 (Figure 3). Predicted amino acid components of recA in UPTC NCTC12894 and CF89-12 showed comparable composition, characterised by higher molar concentrations of alanine, glycine, isoleucine, leucine and lysine, similar to that of *C. lari* JCM2530 and *C. jejuni* 81-176 (Table 3). Composition prediction for recA proteins also indicated that cysteine and tryptophane would be present in one to three residues in the three UPTC strains and *C. lari*, as well as in *C. jejuni* and *C. fetus* (Table 3).

Table 2. Possible ORF of the *recA* gene in UPTC, *C. lari* and other campylobacters

Campylobacter	ORF	Amino acid	CMW	G+C content (%)
UPTC NCTC12894	1035	345	37 314	36.71
UPTC CF89-12	1032	344	37 200	36.92
C. lari JCM2530	1032	344	37 161	36.53
C. jejuni 81-176[5]	1029	343	37 015	36.35
C. fetus 23D[14]	1035	345	37 209	39.13

ORF: open reading frame
CMW: calculated molecular weight

Overlapping, unrelated genes have been described previously in *C. jejuni*,[18,19] and the 3′ terminal region of the *recA* gene in *C. jejuni* and *C. fetus* has been reported to contain a 5′ terminal region of the putative enolase gene.[5,15] Although the *recA*FL-r primer sequence constructed in the present study contained a start codon for the putative enolase gene and a putative ribosome binding site, no further analysis of the enolase gene was carried out.

In conclusion, this study presents nucleotide sequence data on *recA* for *C. lari* and UPTC, which may aid in the phylogenetic positioning of the UPTC group within the genus *Campylobacter*, and in discriminating isolates of the UPTC group. □

Table 3. Predicted amino acid composition of recA in UPTC, *C. lari* and other campylobacters

Amino acid	UPTC NCTC12894	UPTC CF89-12	*C. lari* JCM2530	*C. jejuni* 81-176[5]	*C. fetus* 23D[14]
Asn	12 (3.48%)	12 (3.49%)	11 (3.20%)	12 (3.50%)	11 (3.19%)
Asp	25 (7.25%)	26 (7.56%)	27 (7.85%)	25 (7.29%)	28 (8.12%)
Thr	19 (5.51%)	19 (5.52%)	19 (5.52%)	18 (5.25%)	16 (4.64%)
Ser	17 (4.93%)	17 (4.94%)	18 (5.23%)	18 (5.25%)	22 (6.38%)
Gln	12 (3.43%)	12 (3.49%)	12 (3.49%)	12 (3.50%)	11 (3.19%)
Glu	26 (7.53%)	25 (7.27%)	25 (7.27%)	27 (7.87%)	25 (7.25%)
Pro	10 (2.90%)	10 (2.91%)	10 (2.91%)	9 (2.62%)	9 (2.61%)
Gly	36 (10.43%)	36 (10.47%)	36 (10.47%)	38 (11.08%)	37 (10.72%)
Ala	30 (8.70%)	30 (8.72%)	29 (8.43%)	27 (7.87%)	27 (7.83%)
Val	22 (6.38%)	22 (6.40%)	22 (6.40%)	26 7.58%)	26 (7.54%)
Met	9 (2.61%)	9 (2.62%)	9 (2.62%)	9 (2.62%)	10 (2.90%)
Ile	31 (8.99%)	31 (9.01%)	31 (9.01%)	28 (8.16%)	30 (8.70%)
Leu	28 (8.12%)	28 (8.14%)	28 (8.14%)	28 (8.16%)	27 (7.83%)
Tyr	7 (2.03%)	7 (2.03%)	7 (2.03%)	7 (2.04%)	7 (2.03%)
Phe	10 (2.90%)	10 (2.91%)	10 (2.91%)	9 (2.62%)	10 (2.90%)
His	4 (1.16%)	4 (1.16%)	4 (1.16%)	4 (1.17%)	4 (1.16%)
Lys	31 (8.99%)	29 (8.43%)	31 (9.01%)	30 (8.75%)	31 (8.99%)
Arg	13 (3.77%)	14 (4.07%)	12 (3.49%)	13 (3.79%)	12 (3.48%)
Cys	2 (0.48%)	2 (0.58%)	2 (0.58%)	2 (0.58%)	1 (0.29%)
Trp	1 (0.29%)	1 (0.29%)	1 (0.29%)	1 (0.29%)	1 (0.29%)

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