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Molecular identification and characterisation of catalase and catalase-like protein genes in urease-positive thermophilic *Campylobacter* (UPTC)

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ABSTRACT

Background: Thermophilic *Campylobacter* are important bacterial pathogens of foodborne diseases worldwide. These organisms' physiology requires a microaerophilic atmosphere. To date, little is known about the protective catalase mechanism in urease-positive thermophilic campylobacters (UPTC); hence, it was the aim of this study to identify and characterise catalase and catalase-like protein genes in these organisms.
Materials and methods: Catalase (*katA*) and catalase (Kat)-like protein genes from the Japanese UPTC CF89-12 strain were molecularly analysed and compared with *C. lari* RM2100 and other *C. lari* and thermophilic *Campylobacter* reference isolates.
Results: A possible open reading frame of 1,422 base pairs, predicted to encode a peptide of 474 amino acid residues, with calculated molecular weight of 52.7 kilo Daltons for *katA,* was identified within UPTC CF89-12. A probable ribosome binding site, two putative promoters and a putative ρ-independent transcription terminator were also identified within *katA*. A similar *katA* cluster also existed in the *C. lari* RM2100 strain, except that this strain carries no DcuB genes. However, the Kat-like protein gene or any other homologue(s) were never identified in the *C. lari* RM2100 strain, or in *C. jejuni* and *C. upsaliensis*.
Conclusions: This study demonstrates the presence of catalase/catalase-like protein genes in UPTC organisms. These findings are significant in that they suggest that UPTC organisms have the protective genetic capability of helping protect the organisms from toxic oxygen stress, which may help them to survive in physiologically harsh environments, both within human and animal hosts, as well as in the natural environment.

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Introduction

Oxidative stress is an important factor for organisms employing oxygen as a terminal electron acceptor, since the combination of oxygen and electrons can yield reactive oxygen species, such as superoxide, hydroxyl radicals, hydrogen peroxide.[1] These can lead to damage to proteins, nucleic acids and biomembranes.[2] Reactive oxygen species are also produced by the immune system to kill invading microbes.[2] Thus, bacterial pathogens must resist such reactive oxygen stress encountered, both in the host and in the environment, for their survival. In addition, recent studies indicated that bacteria contain a wide range of enzymes for oxidative stress defence.[3] Catalase is a common enzyme occurring in nearly all living organisms that are exposed to oxygen and catalyses the decomposition of hydrogen peroxide to water and oxygen ($2H_2O_2 \rightarrow 2H_2O + O_2$).[4]

Many bacteria show increased expression of catalase upon incubation with H_2O_2, and an adaptive response makes the bacteria more resistant to subsequent H_2O_2 challenge.[1] In addition, Chelikani et al. [4] categorised that catalases can be divided into three types, namely mono-functional catalases, bi-functional catalase-peroxidases and manganese-containing (non-haeme) catalases.

Thermophilic *Campylobacter* species, primarily *Campylobacter jejuni* and *Campylobacter coli* are curved, Gram-negative organisms and are the most commonly recognised causes of acute bacterial diarrhoea in the Western world.[5–7] *Campylobacter lari* organisms were first isolated in the 1980s, particularly from seagulls.[5,6] *C. lari* has also been shown occasionally to be a cause of clinical infection.[8–10] In addition, an atypical group of urease-positive thermophilic *Campylobacter* (UPTC) organisms have also been isolated from the natural environment in England.[11] Thereafter, these UPTC organisms were described as a biovar or variant of *C. lari*.[12–15] Subsequent isolates were reported in Europe [16–20] and in Japan.[19,20] Thus, at least, these two representative taxa, namely

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urease-negative (UN) *C. lari* and UPTC occur within the *C. lari* species.[21]

Regarding catalase in *Campylobacter*, Grant and Park [22] have already reported that *C. jejuni* and *C. coli* express a single catalase, designated *katA*. In addition, Day et al. [23] showed the importance of catalase of *C. jejuni* in intracellular survival. Regarding oxidative stress defence in *C. lari*, recently, Miller et al. [24] identified one catalase (*katA*) gene in the UN *C. lari* RM2100 strain, following the complete genome sequence analysis (NC_012039). However, no descriptions have yet appeared about this. In addition, no identification has been demonstrated to date as to any counterpart(s) in the UPTC organisms.

We hypothesise that UPTC organisms also contain catalase and catalase-like protein genes, within their genomes. Therefore, the aim of this study was to:

(1) identify and describe *katA* and catalase (Kat)-like protein genes and their homologue(s) in the UPTC CF89-12 strain,
(2) compare these with those genes found in *C. lari* RM2100,
(3) compare the Kat-like protein in the UPTC CF89-12 strain with those from more than 20 *C. lari* isolates, both genotypically and phenotypically.

Materials and methods

Description of organisms employed

The Japanese strain, UPTC CF89-12, which has been isolated from the water of the Asahigawa River, Okayama prefecture, Japan [19], was employed for the construction of a genomic DNA library, cloning, sequencing and characterisation of *katA* and Kat-like protein genes and their adjacent genetic loci. Some other *Campylobacter* reference strains used in the present study are shown in Tables 1 and 2. We also analysed the *katA* Kat-like protein genes and their adjacent genetic loci from other thermophilic *Campylobacter* references strains, accessible in the DDBJ/EMBL/GenBank shown in Tables 1 and 2. A total of 27 *C. lari* isolates ($n = 13$ for UPTC; $n = 14$ for UN *C. lari*) were employed for PCR amplification of the Kat-like protein gene in the present study had also previously been described by Tazumi et al. [25]

Isolates were cultured on Mueller-Hinton agar (Oxoid, Basingstoke, Hampshire, UK) 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 produced by 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, at 37 °C for 48 h.

Genomic DNA preparation

Genomic DNA was prepared using sodium dodecyl sulphate and proteinase K treatment, phenol–chloroform extraction and ethanol precipitation,[26] and adjusted to approximately 500 ng/µL.

Construction of the genomic DNA library of UPTC CF89-12 strain and nucleotide sequence determination

A genomic DNA library was constructed using NEBNext™ DNA Sample Prep. Reagent Set 1 (New England BioLabs Japan Inc., Tokyo, Japan) with the UPTC CF89-12 strain. The DNA was fragmented using Covaris S-Series (Covaris Inc., MA, USA) and 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). The nucleotide sequence (sequence reads 75 bp) was determined using Genome Analyzer IIx and Sequencing Kit (Illumina Inc.). *De novo* assembly of the paired-end reads (35 bp) was carried out using Edena (V2.1.1., http://www.genomic.ch/edena.php) and Velvet (V0.7.11, http://www.ebi.ac.uk/~zerbino/velvet/).

Primer design, PCR amplification, agarose gel electrophoresis of the amplicons and nucleotide sequencing of the katA gene and the Kat-like protein gene

We designed three novel PCR primer pairs, ClakatA-F/-R, ClaKat-like-F/-R and ClaKat-like-318F/+138R, [ClakatA-F, nucleotide positions (np) 1,344–1,363 bp; ClakatA-R, np 1,818–1,837 bp, AB736173; ClaKat-like-F, np 7,144–7,163 bp; ClaKat-like-R, np 7,947–7,966 bp, AB736169; ClaKat-like-318F, np 6,788–6,807 bp; ClaKat-like + 138R, np 8,236–8,255 bp, AB736169], *in silico*, for amplification of the putative *katA* and Kat-like protein genes segments with *C. lari* isolates based on the sequence information of the *katA* and Kat-like protein genes (972 bp) from UPTC CF89-12. It is expected that the primer pair of ClaKat-like-F/-R would generate an amplicon of 823 bp in length of the Kat-like protein gene segments with *C. lari* isolates. In addition, the ClaKat-like-318F/+138R primer pair might be expected to generate the putative full-length Kat-like protein gene containing approximately 1,500 bp segment in length.

Nucleotide sequence alignment analysis was performed to design the primer pair using CLUSTAL W software (1.7 program) [27] incorporated in the DDBJ. The PCR mixture contained 30 ng of the whole genomic DNA, 10 mmol/L Tris-HCl, pH 8.3, 50 mmol/L KCl, 1.5 mmol/L $MgCl_2$, 400 µmol/L of each dNTP, 1 µmol/L of each primer and a total of 1 unit of EX Taq DNA polymerase (TaKaRa Bio Inc., Tokyo, Japan). The PCR was performed in 50 µl volume at 94 °C for 3.0 min, for 30 cycles at 94 °C for 1.0 min, at 55 °C for 1.0 min, at 72 °C for 1.5 min and, finally, at 72 °C for 10 min. Amplified PCR products were

Table 1. Thermophilic Campylobacter isolates analysed in the present study and their accession numbers of nucleotide sequence data accessible in the DDBJ/EMBL/GenBank.

Isolate no./organism		Source	Country	Accession number	Kat-like protein gene
1.	UPTC NCTC12892	River water	England	NA	–
2.	UPTC NCTC12894	Sea water	England	NA	+
3.	UPTC CF89-12	River water	Japan	AB736173	+
4.	UPTC CF89-14	River water	Japan	NA	+
5.	UPTC A1	Seagull	N Ireland	AB827927	+
6.	UPTC 89049	Human	France	AB827928	+
7.	UPTC 92251	Human	France	AB827929	+
8.	UPTC 27	Mussel	N Ireland	NA	+
9.	UPTC 136	Scallop	N Ireland	NA	+
10.	UPTC 150	Cockle	N Ireland	NA	–
11.	UPTC 182	Sea water	N Ireland	NA	–
12.	UPTC 476	Mussel	N Ireland	NA	–
13.	UPTC 504	Mussel	N Ireland	NA	–
14.	UN *C. lari* JCM2530^T	Seagull	Japan	NA	–
15.	UN *C. lari* 28	Mussel	N Ireland	AB827930	–
16.	UN *C. lari* 170	Seagull	Japan	NA	–
17.	UN *C. lari* 175	Seagull	Japan	NA	–
18.	UN *C. lari* 176	Black-tail gull	Japan	NA	–
19.	UN *C. lari* 264	Mussel	N Ireland	NA	–
20.	UN *C. lari* 274	Mussel	N Ireland	NA	–
21.	UN *C. lari* 295	Human	Canada	NA	–
22.	UN *C. lari* 298	Human	Canada	NA	–
23.	UN *C. lari* 299	Human	USA	NA	–
24.	UN *C. lari* 300	Seagull	USA	NA	–
25.	UN *C. lari* 382	Mussel	N Ireland	NA	–
26.	UN *C. lari* 448	Mussel	N Ireland	NA	–
27.	UN *C. lari* 84C-1	Human	N Ireland	AB827931	–
28.	UN*C. lari* RM2100	Human	USA	NC_012039	(–)
29.	*C. coli* RM2228	Chicken	USA	NZ_AAFL01000000	(+)
30.	*C. coli* JV20	Human	USA	NZ_AEER01000000	(+)

Notes. N, Northern; NA, not available; –, negative; +, positive.

Table 2. Sequence similarities (%) of the nucleotide (upper right) and amino acids (lower left) of the Kat-like protein and katA genes.

	Organism	1	2	3	4	5	6	7	8	9	10	11
1.	*C. lari* UPTC CF89-12		96.2	96.3	96.2	91.1	91.8	62.4	33.4	30.5	30.7	27.7
2.	*C. lari* UPTC A1	96.9		99.6	99.7	90.8	91.5	58.4	46.2	47.3	47.0	45.4
3.	*C. lari* UPTC 89049	97.2	99.6		99.6	90.9	91.6	58.7	47.3	47.3	47.1	46.4
4.	*C. lari* UPTC 92251	96.9	99.7	99.6		90.8	91.5	58.7	47.4	47.2	46.9	44.3
5.	*C. coli* RM2228	92.9	93.8	94.1	93.8		98.5	64.3	28.1	33.1	32.6	28.6
6.	*C. coli* JV20	93.8	94.7	95.0	94.7	99.1		64.2	36.4	33.1	32.6	26.3
7.	*H. pylori* P12	59.6	58.4	58.7	58.7	58.4	58.4		29.8	29.4	29.3	22.5
8.	*C. lari* UPTC CF89-12	6.7	17.1	17.1	17.1	6.7	6.7	5.5		82.9	82.7	60.0
9.	*C. coli* RM2228	6.5	16.5	16.5	16.5	8.2	8.2	5.3	87.8		99.1	57.0
10.	*C. coli* JV20	6.5	16.5	16.5	16.5	8.2	8.2	5.1	87.6	99.6		57.2
11.	*H. pylori* P12	7.7	21.5	21.5	21.5	7.7	7.7	5.5	53.2	52.9	52.4	

C., Campylobacter; *H., Helicobacter*; Isolate no. 1–7, Kat-like protein gene; 8–11, katA gene

separated by 0.8% [w/v] agarose gel electrophoresis in 0.5 × TBE at 100 V and detected by staining with ethidium bromide. The PCR products were purified using a QIA quick PCR purification Kit (QIAGEN) and the purified fractions were subjected to cycle sequencing with BigDye terminator (version 3.1; Applied Biosystems, Tokyo, Japan) and with sequencing primers. The reaction products were separated and detected with ABI PRISM™ 3100 Genetic Analyzer (Applied Biosystems Ltd, Paisley, Scotland, UK).

Nucleotide sequence analyses of the putative katA and Kat-like protein genes and their adjacent genetic loci and deduced amino acid sequence alignment analyses of the possible open reading frames

Nucleotide sequence analyses of the putative *katA* and Kat-like protein genes and their adjacent genetic loci and deduced amino acid sequence alignment analysis of the possible open reading frame (ORF) of the Kat-like protein gene were carried out using the GENETYX-Windows computer software version 9 (GENETYX Co., Tokyo, Japan).

Catalase activity determination of the C. lari isolates

Catalase activity was determined using the Catalase Activity Assay Kit (Cayman Chemical Company, Ann Arbor, MI, USA), according to the manufacturer's instructions. Briefly, Cayman's Catalase Assay Kit utilises the peroxidatic function of CAT for determination of enzyme activity. The method is based on the reaction of the enzyme with methanol in the presence of an optimal concentration of H_2O_2. The formaldehyde produced is measured colorimetrically

with 4-amino-3-hydrazino-5-mercapto-1,2,4-triazole (Purpald) as the chromogen. Purpald specifically forms a bicyclic heterocycle with aldehydes, which upon oxidation changes from colourless to a purple colour.

Results

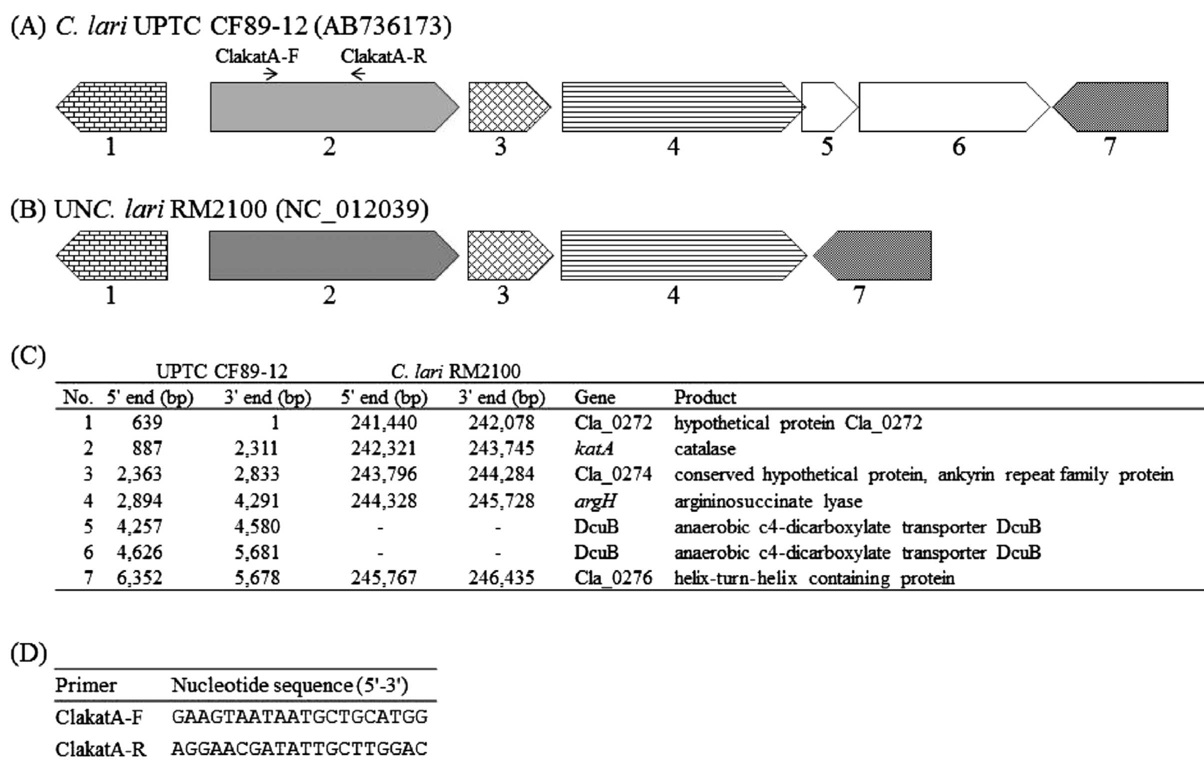
Schematic representations of the putative catalase genes, katA and their adjacent genetic loci within the UPTC CF89-12 and C. lari RM2100 strains genomic DNA following nucleotide sequencing and sequence analyses

Putative *katA* genes and their adjacent genetic loci within the UPTC CF89-12 (AB736173) and *C. lari* RM2100 strains are schematically shown in Figure 1. In the present study, we identified a possible ORF [1,422 bp, np 887–2,308 bp] for the *katA* in the UPTC CF89-12 strain (Figure 1(A)). This was predicted to encode a peptide of 474 amino acid residues with the calculated molecular weight (CMW) of 52.7 kilo Daltons (kDa). The possible ORF of the gene in the UPTC CF89-12 strain was identified, based on the comparison of nucleotide and deduced amino acid sequence similarities with that of the corresponding gene in the *C. lari* RM2100 strain. Probable ribosome-binding (RB) site (Shine-Dalgarno sequence) [28] that is complementary to a highly conserved sequence of CCUCCU close to the 3′-end of 16S ribosomal RNA, AGGAGA (np 877–882 bp) for the *katA* gene was identified in the UPTC CF89-12. We identified the putative promoter structure, consisting of a consensus sequence at the −10 -like region (TATAAT; np 841–846 bp) upstream of the *katA* gene in the UPTC CF89-12 strain, as well as the start codon ATG (np 887–889 bp) (Figure 2(A)). However, no consensus sequences at the −35 -like region were identified, and a semi-conserved T-rich region (np 814–827 bp; T, 11/14) was identified instead of the region (Figure 2(A)), as shown in *Rpo*D promoters in the *C. jejuni* genome.[29]

A hypothetical protein gene [conserved hypothetical protein gene (a structural gene for ankyrin repeat family protein; np 2,363–2,833 bp)], argininosuccinate lyase (*argH*; np 2,894–4,291 bp), two anaerobic c4-dicarboxylate transporter DcuB genes (np 4,257–4,580 bp and np 4,626–5,681 bp) and helix-turn-helix containing protein gene (np 6,352–5,678 bp) also occurred downstream of the *katA* gene in this direction in the UPTC CF89-12 strain.

Schematic representation of the Kat-like protein gene and its adjacent genetic loci within the UPTC CF89-12 strain

We then identified a putative Kat-like protein structural gene (np 7,126–8,097 bp; No. 10 gene in Figure 4(A); AB736169) within the UPTC CF89-12 based on the comparison of the nucleotide and deduced amino acid sequence similarities, with those of the corresponding gene in the *C. coli* RM2228 and JV20 strains (NZ_AAFL00000000; NZ_AEER00000000), as schematically represented in Figure 4(A). This was predicted to encode a peptide of 323 amino acid residues with the CMW of



(C)

No.	UPTC CF89-12 5' end (bp)	UPTC CF89-12 3' end (bp)	*C. lari* RM2100 5' end (bp)	*C. lari* RM2100 3' end (bp)	Gene	Product
1	639	1	241,440	242,078	Cla_0272	hypothetical protein Cla_0272
2	887	2,311	242,321	243,745	*katA*	catalase
3	2,363	2,833	243,796	244,284	Cla_0274	conserved hypothetical protein, ankyrin repeat family protein
4	2,894	4,291	244,328	245,728	*argH*	argininosuccinate lyase
5	4,257	4,580	-	-	DcuB	anaerobic c4-dicarboxylate transporter DcuB
6	4,626	5,681	-	-	DcuB	anaerobic c4-dicarboxylate transporter DcuB
7	6,352	5,678	245,767	246,435	Cla_0276	helix-turn-helix containing protein

(D)

Primer	Nucleotide sequence (5'-3')
ClakatA-F	GAAGTAATAATGCTGCATGG
ClakatA-R	AGGAACGATATTGCTTGGAC

Figure 1. Schematic representations of the putative catalase gene (*katA*) and its adjacent genetic loci in UPTC CF89-12 (A) and UN *C. lari* RM2100 (B). Summaries of those in the two strains (C) and locations of the novel PCR primer (A) designed *in silico* and their sequences (D) were also shown. – absent.

(A)

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                                                                        T-rich region
C. lari RM2100  242,211 ATTTCATAATCATTTTTAAATAATGATAATTTTTATCGTTTAATTTTATTTTAATAATACAGGG 242,275
UPTC CF89-12        777 --..ATACC.GGA.C..C.............................................T....    839

                        -10 region                                RBS     Start codon
C. lari RM2100  242,276 ATATAATTTATCAAATAATAAAAATTATTATCT--AAAGGAGACACAATGAAAAAAT 242,330
UPTC CF89-12        840 ................................T.AA........T............C     896
```

(B-1)

```
                                     Stop codon  Transcriptional terminator
C. lari RM2100  243,726 AAAAAGCTTTAAAAATGTAATTTTTACTTGCCACTTT-CGGGTGGCAAATTAAAGATTATAAAGGAAAAATATGTTTAGC 243,804
UPTC CF89-12      2,292 .....................................AAAA........C..............................   2,371
```

(B-2)

```
              A
       T            A

      T              A

         T        A
          C ------ G
          A ------ T
          C ------ G
          C ------ G
          G ------ C
          T ------ A
AATTTTTACT ------ AACTAAAGA
          ↑        ↑
        2,319    2,339
```

Figure 2. Nucleotide sequence alignment analyses of the putative promoter structures, consisting of a semi-conserved T-rich region and a −10 like region, as well as the start codon ATG, upstream of the *katA* gene (A). Promoter structures, RB site and start codon in the UN *C. lari* RM2100 strain are overlined. Dots indicate identical bases; changes are so indicated; dashes are deleted bases; positions identical in all isolates are marked by asterisks; numbers at the left and right refer to bp of the nucleotide sequences of the isolates accessible in DDBJ/EMBL/GenBank, respectively (B-1). Cases of putative intrinsic ρ-independent transcriptional terminator structures downstream of the stop codon for the *katA* gene cluster, downstream of the *argH* gene in UPTC CF89-12 and *C. lari* RM2100 strains and (B-2) the secondary structure for the terminator in the UPTC CF89-12.

```
UPTC CF89-12 (small)        1 MQKSFWIKIRHKINGCYFILEFFIIFSMIAIGG-RYGGIGLGVAGGLGMCILVLVFGMQPASLPVSVVFIILAVITCVSVLQSIGGLDLLVKIAEKILKK  99
UPTC CF89-12 (large)       0 ----------------------------------------------------------------------------------------------------   0
C. lari RM2100              1 .DLMIILQLIVFL-.AI.------------...I.L...AI.Y.....VVV.G..LMKPGNIPWDVILI.AA.IAAI-.AM.QA....YM...T.RV.R.  86
A. salmonicida LFI1238      1 .NID..LQLAVVL-..IG------------...-.F..V...A.....V.L...G..L....P..T.LL..I...A.T....GA....F..R....L.R.  86

UPTC CF89-12 (small)      100 KPQAIVFM---------------------------------------------------------------------------------------------- 107
UPTC CF89-12 (large)        1 ------------------------VAFSVYPVIAEVAAQAKIRPERPFSVSVIAAGIGVVSSPMSAATAGMVAILAVSGVGLVQILAV-VMPAFLIGVVC  75
C. lari RM2100             87 H.KF.NYLA.ACGWLL..LA..GNAV..LM..VID..KSQN.K.SA.L.LM.V.SQ..ITA..V...VVY.TGV.EPL.WSYPALIGIWIVTT.IACMAT 186
A. salmonicida LFI1238     87 ..S..T.LG..I..M.V.....S....A..........E........M.MA...S..AITA..........I.S.SLYD.SFF.....-S.......AL. 185

UPTC CF89-12 (large)       76 ACLSVFKRGKELEQDEEFQRRVKAGEYQFLTQDKQEDKQEDKQEDKQEDKQEDKQEADPKVKRALLIFALGILTIIFFGTFTNLLPHYELANGKIERLST 175
C. lari RM2100            187 .FIVSLITPMD.SK.PVY.E.L...----.VKSASDVL.SE--------------DK.GA.LSVA..LITV.CVVLYA.--AISNNIKWIDPVVIP--R 263
A. salmonicida LFI1238    186 G.AF.Y...L.....P......AS....LIHE.VNSPEE.Y.PST-------------G..ASV.V.G..VA.V.L....KS...SWDI-D..TVV... 270

UPTC CF89-12 (large)      176 PNLIQMIMLATACLIMLFAKVPANKLGEASVFKSGLIGVVGVFGIAWMTGTFFEAYKPLFSDSLSHIVEDYPYLFGVALFAFSMVIFSPAATVAALMP-- 273
C. lari RM2100            264 DAA.MSFL.TA.T..TFLC..EPA.ILNT......MTAC.C...V..LGN..VAGHEAGIKEVAGEW.KQT.AMLA..F.FA..LLY.Q...AK.IV.VI 363
A. salmonicida LFI1238    271 .M....V...S.LF.IV.....SD.FASG...R............S.......N..Q.F.ETTFEGM..TA.MM..LI..C..A.....S...S....-- 368

UPTC CF89-12 (large)      274 ---LGVSLGIPPQ--ILIVLYPCVSGDFIVPG-ANQIACVAFDRTGTTKIGKFVINHSYLRPGFALIIGAVVSAYFISQIVFSY 351
C. lari RM2100            364 IAA..I.ATN.SDSYM.VACFAA..AL.VL.TYPTLLGA.QM.D....R....IF..AFFI..VLA.AI..ALGFLAVGML--- 445
A. salmonicida LFI1238    369 ---..AAM....G--L.VA...ATC.......-TG..G.LS.......RL.TY......IL...VMV.SS.TAG....K...-- 445
```

Figure 3. Amino acid sequence alignment analysis of the possible DcuB genes ORFs in the UPTC CF89-12 (the first small and the second large) and *C. lari* RM2100 strains. Amino acid sequence of *A. salmonicida* LFII238 was also aligned for comparison.

35.9 kDa. Probable RB site, AGGAGC (np 7,116–7,121 bp), two putative promoters at the −35 (TTGATA; np 7,060–7,065 bp) and −10 (TACAAT; np 7,091–7,096 bp)-like structures immediately upstream of the Kat-like protein gene (Figure 5(A)). In addition, a hypothetically intrinsic ρ-independent transcriptional terminator was also identified (np 8,105–8,119 bp) (Figure 5(B)).

Sequence similarities of the nucleotide and deduced amino acid of the Kat-like protein and katA genes

As shown in Table 2, the nucleotide sequence and the deduced amino acid sequence similarities of the possible ORFs were examined for the Kat-like protein genes

(A) *C. lari* UPTC CF89-12 (AB736169)

(B) UN*C. lari* RM2100 (NC_012039)

(C)

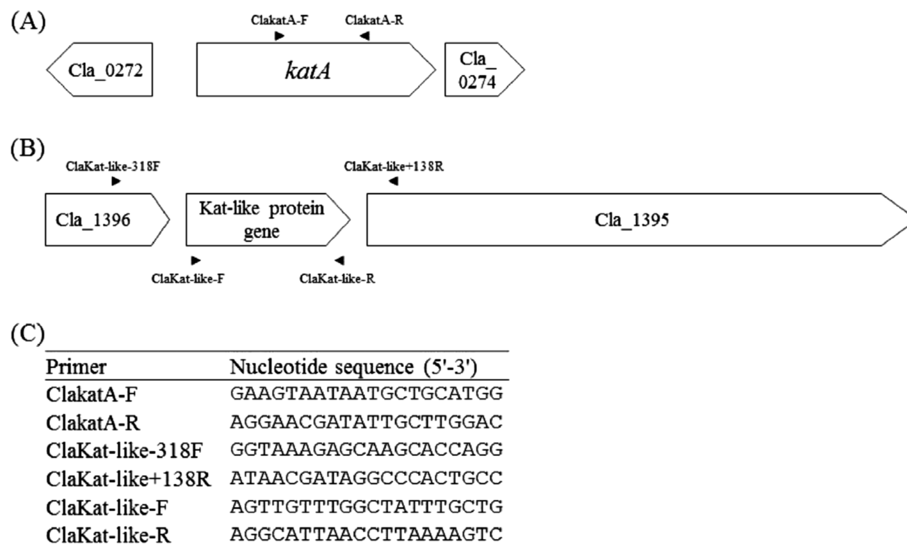
No.	*C. lari* RM2100 5' end (bp)	*C. lari* RM2100 3' end (bp)	UPTC CF89-12 5' end (bp)	UPTC CF89-12 3' end (bp)	Gene	Product
1	1,351,016	1,351,930	915	1	*ilvE*	branched-chain amino acid aminotransferase
2	1,350,918	1,350,712	1,013	1,219	NA	4-oxalocrotonate tautomerase family protein
3	1,350,715	1,350,254	1,216	1,677	*bcp*	bacterioferritin comigratory protein (AhpC/Tsa family)
4	1,350,260	1,349,187	1,671	2,744	NA	conserved hypothetical protein (DUF208 domain protein)
5	1,349,118	1,348,678	2,813	3,253	*fabZ*	(3R)-hydroxymyristoyl-ACP dehydratase
6	1,348,678	1,347,887	3,250	4,044	*lpxA*	UDP-N-acetylglucosamine acyltransferase
7	1,347,894	1,346,659	4,037	5,266	*clpX*	ATP-dependent protease ATP-binding subunit ClpX
8	1,346,662	1,345,625	5,296	6,306	*mreB*	rod shape-determining protein MreB
9	1,345,635	1,344,895	6,296	7,036	NA	rod shape-determining protein MreC
10	-	-	7,126	8,097	NA	catalase-like protein
11	1,344,813	1,341,550	8,197	11,460	*carB*	carbamoyl phosphate synthase large subunit
12	1,341,553	1,341,131	11,457	11,879	NA	hypothetical protein
13	1,340,096	1,341,130	12,910	11,876	*ansB*	L-asparaginase

-, no exsistence; NA, not available

Figure 4. Schematic representations of the putative Kat-like protein gene and its adjacent genetic loci in UPTC CF89-12 (A) and its corresponding region in UN *C. lari* RM2100 (B) strains. –, negative: NA, not available (C) lari RM2100 (B) and (C) description of 13 genes in both strains, indicating their position and funtionality. –, negative; NA, not available.

Figure 5. Nucleotide sequence alignment analyses of the putative promoter structure, as well as the start codon and RBS (A) and the hypothetically intrinsic ρ-independent transcriptional terminator structure (B), for the Kat-like protein gene from the UPTC CF89-12 and three other UPTC strains, UPTC 92251, A1 and 89049.

within UPTC CF89-12 and some other related bacteria. Regarding the Kat-like protein gene, high nucleotide (90.8–99.7%) and deduced amino acid (92.9–99.7%) sequence similarities of the putative gene are shown between the UPTC CF89-12 and three UPTC isolates and *C. coli* RM2228 and JV20 strains, as shown in Table 2. Similarity comparisons were also carried out with bacterial *katA* genes (Table 2). In addition, as shown in Table 2,



Primer	Nucleotide sequence (5'-3')
ClakatA-F	GAAGTAATAATGCTGCATGG
ClakatA-R	AGGAACGATATTGCTTGGAC
ClaKat-like-318F	GGTAAAGAGCAAGCACCAGG
ClaKat-like+138R	ATAACGATAGGCCCACTGCC
ClaKat-like-F	AGTTGTTTGGCTATTTGCTG
ClaKat-like-R	AGGCATTAACCTTAAAAGTC

Figure 6. A schematic representation of the *katA*(A) and Kat-like protein (B) gene loci identified within the UPTC CF89-12 strain genomic DNA and the locations of the primer pairs for amplification of the genes segment (A and B), and the primer sequences employed (C).

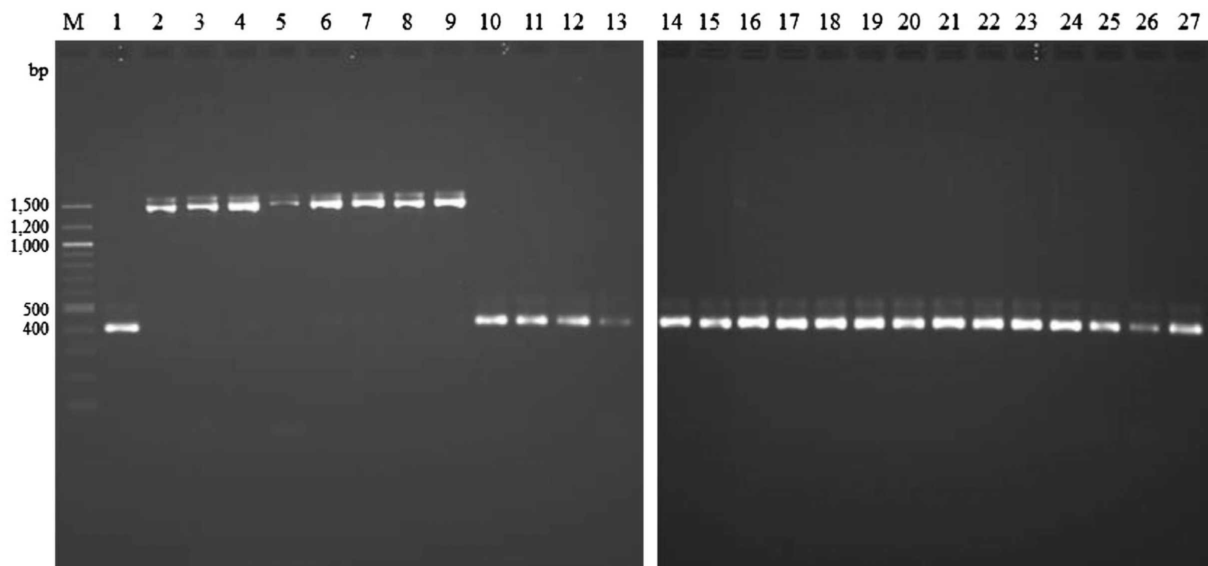


Figure 7. Agarose gel electrophoresis profiles of the Kat-like protein gene segment using the primer pair of ClaKat-like-F/-R. Lane M, 100 bp DNA ladder; Lane 1, UPTC NCTC12892; lane 2, NCTC12894; lane 3, UPTC CF89-12; lane 4, CF89-14; lane 5, UPTC A1; lane 6, UPTC 89049; lane 7, UPTC 92251; lane 8, 27; lane 9, 136; lane 10, 150; lane 11, 182; lane 12, 476; lane 13, 504; lane 14, UN *C. lari* JCM2530^T; lane 15, 28; lane 16, 170; lane 17, 175; lane 18, 176; lane 19, 264; lane 20, 274; lane 21, 295; lane 22, 298; lane 23, 299; lane 24, 300; lane 25, 382; lane 26, 448; lane 27, 84C-1.

these isolates showed very low sequence similarities of both the nucleotides and amino acids between Kat-like protein and *katA* genes.

PCR amplification of the Kat-like protein gene segments, agarose gel electrophoresis of the amplicons and nucleotide sequencing with C. lari isolates

PCR amplification of the Kat-like protein gene segment was performed using the primer pair of ClaKat-like-318F/+138R (Figure 6) with 27 *C. lari* isolates (Table 1; *n* = 13 for UPTC and *n* = 14 for UN *C. lari*). When PCR amplification was carried out on the Kat-like protein gene segments with the *C. lari* isolates, eight isolates (29.6% positive) [*n* = 8 for UPTC (61.5% positive); *n* = 0 for UN *C. lari* (0% positive)] generated an approximately 1,500 bp amplicon out of 28 isolates, respectively (Figure 8). The other 19 isolates generated an approximately 400 bp amplicon (Figure 7). The overall results are summarised in Table 1. Thus, *C. lari* isolates appear to carry the Kat-like protein gene, but not so frequently. In addition, the UPTC organisms carried the Kat-like protein gene, examined in the present study, relatively frequently.

Figure 8. Amino acid sequence alignment analyses of the possible ORF of the Kat-like protein gene from the UPTC CF89-12 strain, some other UPTC isolates and reference *C. coli* RM2228 and *C. coli* JV20 strains (A). Nucleotide sequence alignment analyses of the Kat-like protein gene from the some *C. lari* isolates for comparison (B).

We then, performed deduced amino acid sequence alignment analysis of the possible ORF of the Kat-like protein gene among the four *C. lari* UPTC isolates, UPTC CF89-12, UPTC 89049, UPTC 92251 and UPTC A1 and two *C. coli* RM2228 and JV20 reference strains (Figure 8(A)). Nucleotide sequence alignment analyses of the Kat-like protein gene and its adjacent genetic loci from the UPTC CF89-12 strain and some other *C. lari* isolates were also carried out for comparison (Figure 8(B)).

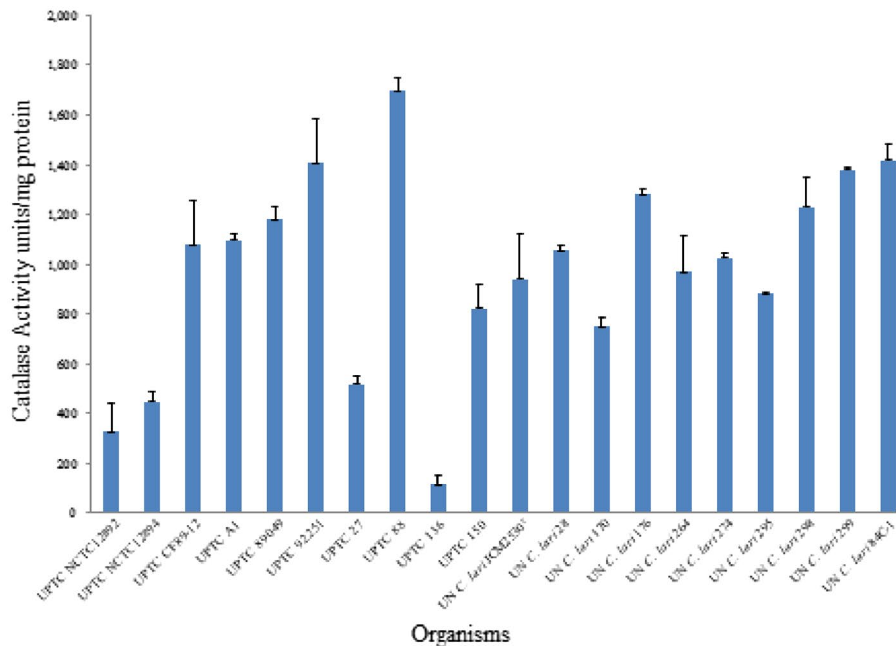


Figure 9. Catalase activities determined using the Catalase Activity Assay Kit.

Table 3. Sequence similarities (%) of the nucleotide (upper right) and amino acid (lower left) of the full-length DcuB gene.

Isolate no.		1	2	3	4
1	UPTC CF89-12		49.2	52.8	53.7
2	*C. lari* RM2100	30.5		49.9	50.2
3	*C. jejuni* M1	31.8	35.6		48.7
4	*A. salmonicida* LFI1238	58.0	34.9	35.2	

Table 4. Summary.

What is known about the subject: • The ability of a bacterium to survive in oxygen-aggressive environments is important to its potential and persistence as a food-poisoning organism • Catalase enzymes are important protective mechanisms aiding the survival of bacteria in harsh oxidative environments
What this paper adds: • Comparative genetics between hitherto unreported catalase genes in UPTC campylobacters and other established species *C. lari* and other established species

Catalase activity determination of C. lari isolates

Figure 9 shows the catalase activities of cell-free extracts from the 20 *C. lari* isolates (n = 10 for UN *C. lari*; n = 10 for UPTC). As shown in Figure 9, the catalase activities were distributed widely ranging from approximately 100 to 1,700 units/mg protein among the 20 *C. lari* isolates.

Discussion

A possible overlap was identified in the 35 nucleotides between the structural *argH* gene (No. 4 gene in Figure 1(A)) and the first small DcuB gene (No. 5 gene in Figure 1(A)). Another overlap of the four nucleotides was also identified between the second large DcuB gene (No. 6 gene in Figure 1(A)) and the Cla_0276 gene (No. 7 gene in Figure 1(A)) in the UPTC CF89-12 strain.

Regarding the two DcuB genes, it is very interesting that both the first DcuB gene (324 bp in length) and the second DcuB gene (1,056 bp in length), that are absent in the *katA* gene and its adjacent genetic loci cluster within the *C. lari* RM2100, occurred between the *argH* gene and the Cla_0276 in the UPTC CF89-12 strain. Based on the nucleotide and amino acid sequences analyses, the first small DcuB gene (np 4,257–4,580 bp) commenced with an ATG start codon (np 4,257–4,259 bp) and terminated with a TGA (np 4,578–4,580 bp) stop codon. The large DcuB gene (np 4,626–5,681 bp) commenced with a GTG (np 4,626–4,628 bp) and terminated with a TAA (np 5,679–5,681 bp) in the UPTC CF89–12 strain (AB736173).

These two DcuB structural genes add up to 1,380 bp in length, and interestingly, the total bp length is equivalent

to the full length of the DcuB structural gene from other thermophilic campylobacters. Therefore, these two DcuB genes may possibly be generated through the mutation, integration, recombination or deletion from an ancestor DcuB gene in the UPTC CF89-12 strain. Thus, the small DcuB gene appears to be a truncated pseudogene and the large DcuB a truncated gene. Amino acid sequence alignment analysis of the possible DcuB genes ORFs in the UPTC CF89-12 (np 4,257–5,681 bp, AB736173) and *C. lari* RM2100 (np 118,366–119,700 bp, NC_012039) isolates, as well as *A. salmonicida* LFII238 (np 767,012–768,346 bp, NC_011312), are shown in Figure 3. Then, we attempted to analyse sequence similarities (%) of the nucleotide and amino acid of the full-length DcuB gene among the bacterial isolates as shown in Table 3.

In the *C. lari* RM2100 strain, some genes encoding integrase and recombinase (locus-tag, Cla_0735, Cla_0824, Cla_0153, Cla_0496 and Cla_0382) have already been detected (NC_012039). These enzymes may be involved in the generation of these two small and large DcuB genes segments within the UPTC CF89-12 genomic DNA. Natural transformation in *Campylobacter* species [30] and widespread gene decay and failure of putative horizontally transferred genes in prokaryotes [31] have already been described.

A putative intrinsic ρ-independent transcriptional terminator structure which contains a G + C-rich region (Figure 2(B-1)) was demonstrated for the *katA* gene cluster downstream of the *argH* gene (No. 4 gene in Figure 1(A)) in the UN *C. lari* RM2100 and UPTC CF89-12 strains. A secondary structure model for the terminator in the UPTC CF89-12 strain was demonstrated in Figure 2(B-2). Thus, the *katA* gene may possibly constitute a cluster with the other two structural genes (Cla_0274 and *argH*) downstream of that in the UPTC CF89-12 strain genomic DNA.

In the present study, we also showed the *katA* gene cluster which consists of the three structural genes, *katA*, conserved hypothetical protein (ankyrin repeat family protein) and argininosuccinate lyase (*argH*) genes in the *C. lari* RM2100 strain, as schematically represented in Figure 1(B). Two putative promoters and a putative transcriptional terminator structure were also seen in the *C. lari* RM2100 strain (Figure 2).

Harris et al. [32] described that the gene immediately downstream of *katA*, encoding a protein proposed to name *katA*-associated protein, KapA (HP0874), has a role in the *in vitro* resistance to hydrogen peroxide in *H. pylori*. In addition, Odenbreit et al. [33] and Harris et al. [32] indicated that *kapA* gene does not influence catalase activity per se.[34] In the present study, the Kat-like protein gene segment of approximately 1,500 bp was amplified with the eight *C. lari* UPTC isolates, UPTC NCTC12894, UPTC CF89-12, CF89-14, UPTC A1, UPTC 89049, UPTC 92251, UPTC 27 and UPTC 136 out of the 27 isolates using the primer pair of ClaKat-like-318/+138, respectively, as shown in Figure 7 and summarised in Table 1. As shown in Figure 9, in *C. lari* organisms, Kat-like protein gene appears not to influence catalase activity in their cells (Table 4).

This work represents an advance in biomedical science, because it shows for the first time the genetic composition of catalase genes and catalase-like protein genes with the UPTC organisms and helps elucidate a potential role for the UPTC organisms in oxygen-rich environments.

Disclosure statement

No potential conflict of interest was reported by the authors.

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References

[1] Storz G, Imlayt JA. Oxidative stress. Curr. Opin. Microbiol. 1999;2:188–194.
[2] Atack JM, Harvey P, Jones MA, et al. The *Campylobacter jejuni* thiol peroxidases Tpx and Bcp both contribute to aerotolerance and peroxide-mediated stress resistance but have distinct substrate specificities. J. Bacteriol. 2008;190:5279–5290.
[3] Atack JM, Kelly DJ. Oxidative stress in *Campylobacter jejuni*: responses, resistance and regulation. Future Microbiol. 2009;4:677–690.
[4] Chelikani P, Fita I, Loewen PC. Diversity of structures and properties among catalases. Cell. Mol. Life Sci. 2004;61:195–208.
[5] Skirrow MB, Benjamin J. '1001' Campylobacters: cultural characteristics of intestinal campylobacters from man and animals. J Hyg. Camb. 1980;85:427–442.
[6] Benjamin J, Leaper S, Owen RJ, et al. Description of *Campylobacter laridis*, a new species comprising the nalidixic acid resistant thermophilic *Campylobacter* NARTC group. Curr. Microbiol. 1983;8:231–238.
[7] Blaser MJ, Taylor DN, Feldman RA. Epidemiology of *Campylobacter jejuni* infections. Epidemiol. Rev. 1983;5:157–176.
[8] Nachamkin I, Stowell C, Skalina D, et al. *Campylobacter laridis* causing bacteremia in an immunosuppressed patient. Ann. Int. Med. 1984;101:55–57.
[9] Martinot M, Jaulhac B, Moog R, et al. *Campylobacter lari* bacteremia. Clin. Microbiol. Infect. 2001;7:96–97.
[10] 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;40:1053–1055.
[11] Bolton FJ, Holt A, Hutchinson DN. Urease-positive thermophilic campylobacters. Lancet. 1985;325:1217–1218.

[12] Mégraud F, Chevrier D, Desplaces N, et al. Urease-positive thermophilic *Campylobacter Campylobacter laridis* variant isolated from an appendix and from human feces. J. Clin. Microbiol. 1988;26:1050–1051.
[13] Owen RJ, Costas M, Sloss L, et al. Numerical analysis of electrophoretic protein patterns of *Campylobacter laridis* and allied thermophilic campylobacters from the natural environment. J. Appl. Bacteriol. 1988;65:69–78.
[14] 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;9:895–897.
[15] Wilson IG, Moore JE. Presence of *Salmonella* spp. and *Campylobacter* spp. in shellfish. Epidemiol. Infect. 1996;116:147–153.
[16] 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.
[17] Kaneko A, Matsuda M, Miyajima M, et al. Urease-positive thermophilic strains of *Campylobacter* isolated from seagulls *Larus* spp. Lett. Appl. Microbiol. 1999;29:7–9.
[18] Matsuda M, Kaneko A, Stanley T, et al. Characterization of urease-positive thermophilic *Campylobacter* subspecies by multilocus enzyme electrophoresis typing. Appl. Environ. Microbiol. 2003;69:3308–3310.
[19] 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;81:608–612.
[20] 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;205:321–324.
[21] Matsuda M, Moore JE. Urease-positive thermophilic campylobacter species. Appl. Environ. Microbiol. 2004;70:4415–4418.
[22] Grant KA, Park SF. Molecular characterization of *katA* from *Campylobacter jejuni* and generation of a catalase-deficient mutant of *Campylobacter coli* by interspecific allelic exchange. Microbiology. 1995;141:1369–1376.
[23] Day WA, Sajecki JL, Pitts TM, et al. Role of catalase in *Campylobacter jejuni* intracellular survival. Infect. Immun. 2000;68:6337–6345.
[24] Miller WG, Wang G, Binnewies TT, et al. The complete genome sequence and analysis of the human pathogen *Campylobacter lari*. Foodborne Pathog. Dis. 2008;5:371–386.
[25] Tazumi A, Kakinuma Y, Moore JE, et al. Demonstration of the absence of intervening sequences within 23S rRNA genes from *Campylobacter lari*. J. Basic Microbiol. 2009;49:386–394.
[26] Harrington CS, Thomson-Carter FM, Carter PE. Evidence for recombination in the flagellin locus of *Campylobacter jejuni*: implications for flagellin gene typing scheme. J. Clin. Microbiol. 1997;35:2386–2392.
[27] 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;22:4673–4680.
[28] Matsuda M, Shigematsu M, Tazumi A, et al. Cloning and structural analysis of the full-length cytolethal distending toxin (cdt) gene operon from *Campylobacter lari*. Br. J. Biomed. Sci. 2008;65:195–199.
[29] Petersen L, Larsen TS, Ussery DW, et al. *Rpo*D promoters in *Campylobacter jejuni* exhibit a strong periodic signal instead of a −35 box. J. Mol. Biol. 2003;326:1361–1372.
[30] Wang Y, Taylor DE. Natural transformation in *Campylobacter* species. J. Bacteriol. 1990;172:949–955.
[31] Liu Y, Harrison PM, Kunin V, et al. Comprehensive analysis of pseudogenes in prokaryotes: widespread gene decay and failure of putative horizontally transferred genes. Genome Biol. 2004;5:R64.
[32] Harris AG, Hinds FE, Beckhouse AG, et al. Resistance to hydrogen peroxide in *Helicobacter pylori*: role of catalase KatA and Fur, and functional analysis of a novel gene product designated 'KatA-associated protein', KapA HP0874. Microbiology. 2002;148:3813–3825.
[33] Odenbreit S, Wieland B, Haas R. Cloning and genetic characterisation of *Helicobacter pylori* catalase and construction of a catalase deficient mutant. J. Bacteriol. 1996;178:6960–6967.
[34] Harris AG, Wilson JE, Danon SJ, et al. Catalase KatA and KatA-associated protein KapA are essential to persistent colonization in the *Helicobacter pylori* SS1 mouse model. Microbiology. 2003;149:665–672.