Construction and expression of a recombinant urease gene cluster from *Campylobacter sputorum* biovar *paraureolyticus*

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ABSTRACT

Recombinant full-length urease gene cluster and seven 100% deletion recombinant variants of urease subunits genes, (*ureG*, *ureH*, *ureA*, *ureB*, *ureC*, *ureE* and *ureF*) were constructed *in vitro* from the *Campylobacter sputorum* biovar *paraureolyticus* LMG17591 strain and expressed in *Escherichia coli* JM109 cells. A urease-positive reaction (1.885 μmol/min/mg protein) in the log-phase cultured *E. coli* cells transformed with pGEM-T vector carrying the recombinant full-length urease genes cluster was detected. Among the seven 100% deletion recombinant variants, each of the *ureG*-, *ureH(D)*-, *ureA*-, *ureB*-, *ureC*-, *ureE*- and *ureF*-deletion variants showed no change in assay of the urease reaction, and similarly as in the *E. coli* cell lysate with pGEM-T vector only. Recombinant full-length urease gene cluster and 100% deletion recombinants of the *ureE* gene in the transformed and log-phase cultured *E. coli* cells from the *C. sputorum* showed positively accelerated urease activities when cultured in the medium containing $NiCl_2$ (750 μmol/L), but no activity was accelerated in the *C. sputorum* cultured in $NiCl_2$. In addition, thiourea (20 mmol/L) completely inhibited urease activities from all *C. sputorum* examined. The putative recombinant urease subunits A and C were immunologically identified by Western blot analysis with polyclonal anti-urease α (A) and β (B), raised against *Helicobacter pylori*.

KEY WORDS: Campylobacter sputorum.
Polymerase chain reaction.
Urease.

Introduction

Many species of bacteria produce urease (urea amidohydrolase; EC3.5.1.5), a nickel-containing metalloenzyme that hydrolyses urea to ammonia and carbamate.[1] *Campylobacter lari* was first described as a nalidixic acid-resistant thermophilic *Campylobacter*;[2] however, in 1985, an atypical organism of urease-positive and nalidixic acid-sensitive thermophilic *Campylobacter* (UPTC) was isolated in England.[3] Thereafter, the characterisation of UPTC as a variant or biovar of *C. lari* has been described.[4,5] After the original description of UPTC had appeared, UPTC organisms have been reported in France,[6] Northern Ireland,[7–10] The Netherlands,[11] England,[12] Japan,[13,14] and then Sweden.[15] This UPTC organism is, thus, an atypical taxon within the genus *Campylobacter* which produces urease,[16] as does *Campylobacter sputorum* biovar *paraureolyticus*.[17]

On *et al*. have described an amended report of *C. sputorum* and revision of its infrasubspecific (biovar) divisions, including *C. sputorum* biovar *paraureolyticus*.[17] They first identified 15 strains obtained from faeces of 14 cattle in England and one case of human diarrhoea in Canada among 44 catalase-negative and urease-positive *Campylobacter* groups and described these as a new *C. sputorum* biovar *paraureolyticus*.[17] This is the second urease-producing taxon other than UPTC within the genus *Campylobacter*.

In addition, recent reclassification of *Bacteroides ureolyticus* as *Campylobacter ureolyticus* com. nov., the third taxon of urease-producing *Campylobacter*, has been described.[18–20]

The authors have already described a putative urease gene operon consisting of six closely spaced and possible open reading frames (ORFs) of two structural (*ureA* and *ureB*) and four accessory (*ureE*, *ureF*, *ureG* and *ureH*) genes from a genomic DNA library constructed with a Japanese UPTC CF89-12 strain.[21] This urease gene operon was approximately 5.1 kbp in length. In addition, the authors have already described the *in vitro* construction of a recombinant full-length urease gene operon from UPTC CF89-12 and its expression of large and 100% deletion variants of the UPTC strain urease gene subunits in *Escherichia coli* cells.[22]

Regarding *C. sputorum* biovar *paraureolyticus* urease, the authors have also described the molecular analysis and characterisation of a putative urease gene operon from *C. sputorum* biovar *paraureolyticus* LMG17591.[23] Within the operon, seven closely spaced and possible ORFs for *ureG*, *ureH(D)*, *ureA*, *ureB*, *ureC*, *ureE* and *ureF* were detected in this order. The urease gene operon transcription in the cells was also confirmed by reverse transcription (RT)-PCR analysis.[23]

However, the authors have not yet attempted to construct

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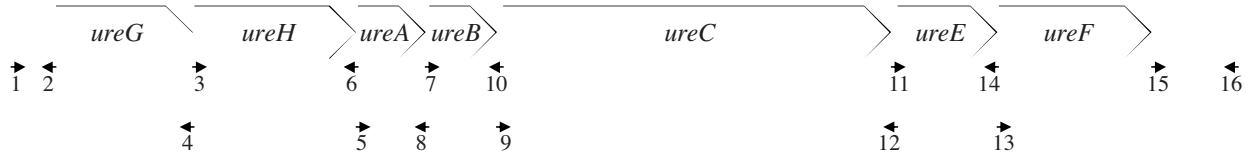
A



B

No.	Primer	Nucleotide sequence (5'-3')	Nucleotide position (bp)
1	ure-F	GGCGAGAGTTTATCATGG	1,008 – 1,025
2	CspuredelG-R	TACTTAATCCTTAATTAAATTTGAGC	1,183 – 1,208
3	CspuredelG-F	ATGAGTGTTTTAAAAACTATACTTAAAGATGG	1,817 – 1,848
4	CspuredelH-R	TGCAAATCTTTAAAAAAGCAG	1,796 – 1,816
5	CspuredelH-F	ATGACACTTACGAAAAAAGAGACAG	2,530 – 2,554
6	CspuredelA-R	TAATTCTCCTTTATAAAATTGG	2,508 – 2,529
7	CspuredelA-F	AAGGAAAATTTTATGAAACTTGGC	2,833 – 2,856
8	CspuredelB-R	CCTTTTAATTTATTGGATTATGG	2,814 – 2,836
9	CspuredelB-F	AATAATGTTTAACATAAGCAGAC	3,161 – 3,183
10	CspuredelC-R	CCTTTCTTATAATTTACCATC	3,136 – 3,156
11	CspuredelC-F	ATGATAGTAAATAAAATTTTAGGC	4,881 – 4,904
12	CspuredelE-R	TTAAAACATATAGTAAAGTT	4,852 – 4,871
13	CspuredelE-F	ATGCTAAGTAGTTTTTTATT	5,324 – 5,343
14	CspuredelF-R	TAGTGATGATGACTCGCATG	5,304 – 5,323
15	CspuredelF-F	CTAACTCATTTTAAATAATCTCGG	6,018 – 6,041
16	ure-R	CAGAAGTAGGCGGTAGA	6,300 – 6,316

Fig. 1. A schematic representation of the putative full-length urease gene cluster and its adjacent genetic loci in *C. sputorum* biovar *paraureolyticus* LMG17591 (A) and the locations of primer pair (ure-F/-R) for the recombinant amplification of the approximately 5.3-kbp urease gene cluster (A) and the primer sequences (B). PCR and I-PCR primer locations to construct seven 100% deletion recombinant variants of the *C. sputorum* biovar *paraureolyticus* urease gene subunits (*ureG*, *ureH*, *ureA*, *ureB*, *ureC*, *ureE* and *ureF*) (A) and primer details, including nucleotide sequences employed (B), are also shown.

an *in vitro* recombinant DNA molecule, which can express catalytically active urease enzyme in *E. coli* by amplifying the urease gene operon from the *C. sputorum* biovar *paraureolyticus*.

Therefore, the aim of this study is first to construct an in vitro recombinant of the full-length urease gene cluster from *C. sputorum* biovar *paraureolyticus* LMG17591 and to express the recombinant urease molecule in *E. coli* cells. Additionally, the authors also aim to construct, *in vitro*, seven 100% deletion recombinant variants of each urease gene subunit, *ureG*, *ureH*, *ureA*, *ureB*, *ureC*, *ureE* and *ureF*, and to express and characterise these in *E. coli* cells, in order to clarify the roles of the accessory genes in *C. sputorum* biovar *paraureolyticus* urease production.

Materials and methods

Bacterial strain and its culture condition

C. sputorum biovar *paraureolyticus* LMG17591 strain, isolated from bovine faeces in Bristol, UK,[17] was used in the present study.[23] The cells were cultured, as described previously.[23]

Genomic DNA preparation

Template DNA was prepared using sodium dodecyl sulphate and proteinase K treatment, phenol-chloroform extraction and ethanol precipitation,[24] and adjusted to approximately 500 ng/μL.

Amplification, construction, extraction and purification

For the amplification of the approximately 5.3-kbp urease gene cluster region from *C. sputorum* biovar *paraureolyticus* LMG17591, consisting of the promoter and terminator regions and seven urease structural and accessory genes, nucleotide position (np) 1008 to 6316 bp (DDBJ/EMBL/GenBank accession number AB479194 for *C. sputorum* biovar *paraureolyticus* LMG17591), the authors designed a polymerase chain reaction (PCR) primer pair, ure-F (np 1008–1025 bp) and ure-R (np 6300–6316 bp) (Fig. 1). The PCR amplification and its product purification were carried out as

Table 1. Details of seven 100% deletion recombinant variants of *C. sputorum* biovar *paraureolyticus* urease gene subunits.

	Deletion	
100% deletion recombinant variant	Nucleotide position (AB479194)	Amino acid residue
ureG-deletion variant	1209–1816	203
ureH-deletion variant	1817–2529	237
ureA-deletion variant	2530–2832	100
ureB-deletion variant	2837–3160	101
ureC-deletion variant	3157–4880	568
ureE-deletion variant	4872–5323	147
ureF-deletion variant	5324–6017	227

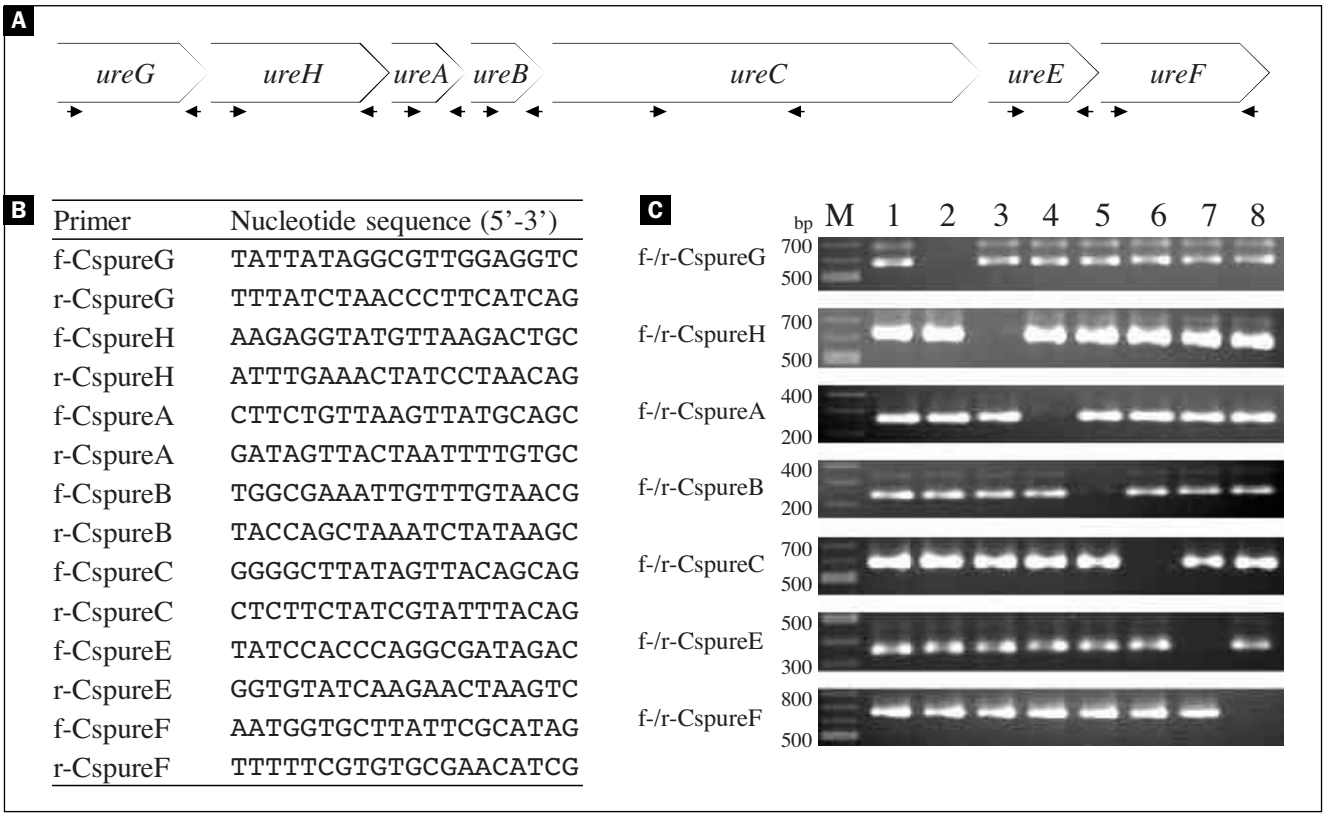


Fig. 2. A schematic representation of the putative full-length seven urease structural and accessory gene cluster identified in *C. sputorum* biovar *paraureolyticus* LMG17591[23] and the locations of seven primer pairs for RT-PCR amplifications (A) and primer details, including nucleotide sequences employed (B). (C) RT-PCR profiles of the transcript segments from each of the seven 100% deletion recombinant variants of the seven urease genes in the *C. sputorum* biovar *paraureolyticus* expressed in the *E. coli* JM109 cells. Lane M: 100-bp DNA ladder (New England BioLabs, Tokyo, Japan). Lane 1: template DNA from recombinant full-length urease gene cluster in the transformed and log-phase cultured *E. coli*; Lane 2: template DNA from the 100% deletion recombinant of the *ureG* gene; Lane 3: *ureH*; Lane 4: *ureA*; Lane 5: *ureB*; Lane 6: *ureC*; Lane 7: *ureE*; Lane 8: *ureF*. Seven primer pairs for each of the RT-PCR employed are shown on the left of the Figure 2C.

described previously.[22] TA cloning of the amplified urease genes cluster region using the pGEM-T vector and its transformation into chemically competent *E. coli* JM109 cells was also performed, as described previously.[22]

The authors then attempted to construct seven 100% deletion recombinant variants of *C. sputorum* biovar *paraureolyticus* LMG17591 urease gene subunits (*ureG*, *ureH*, *ureA*, *ureB*, *ureC*, *ureE* and *ureF*, six urease genes containing-recombinant variants; Table 1) using their specific PCR primer pairs and inverse (I)-PCR procedures with the TA-cloned full-length *C. sputorum* biovar *paraureolyticus* urease gene cluster (Fig. 1). The primer pairs for the I-PCR shown in Figure 1 were designed based on the nucleotide sequence data of the approximately 5.3-kbp full-length urease gene subunit cluster region from *C. sputorum* biovar *paraureolyticus* LMG 17591 strain (AB479194).

The I-PCR was carried out using the KOD-PLUS-Mutagenesis Kit (Toyobo, Osaka, Japan). The I-PCR reaction was performed in 20-µL reaction volumes at 94˚C for 2 min, with 20 cycles at 34˚C for 15 sec, at 50˚C for 30 sec, and at 68˚C for 9 min, followed by a final extension at 68˚C for 10 min.

Amplified I-PCR products were separated by 0.7% (w/v) agarose gel electrophoresis in 0.5×TBE at 100 V and detected by staining with ethidium bromide. The I-PCR products were transformed into chemically competent *E. coli* JM109 cells, as described previously.[22] After transformation, the recombinant plasmids were prepared by an alkaline preparation method. The plasmids were subjected to cycle sequencing with BigDye Terminator (Applied Biosystems, Tokyo, Japan), using the PCR primers or the I-PCR primers. The reaction products were separated and detected on an ABI Prism 3100 genetic analyser (Applied Biosystems).

TA cloning of seven 100% deletion recombinant variants of urease gene subunit, *ureG*, *ureH*, *ureA*, *ureB*, *ureC*, *ureE* and *ureF*, constructed *in vitro* from *C. sputorum* biovar *paraureolyticus* LMG17591, using the pGEM-T vector and their transformation into *E. coli* JM109 cells was also performed, as described above. Details of each 100% deletion are shown in Table 1.

Total cellular RNA was extracted and purified from the transformed and log-phase cultured *E. coli* JM109 cells carrying the recombinant full-length urease gene cluster and seven 100% deletion recombinant variants of the urease gene subunits, *ureG*, *ureH*, *ureA*, *ureB*, *ureC*, *ureE* and *ureF*, from the *C. sputorum* biovar *paraureolyticus*, with RNAprotect Bacteria Reagent and RNeasy Mini Kit (Qiagen, Tokyo, Japan), respectively. For RT-PCR analyses, seven primer pairs shown in Figure 2 were designed *in silico* for the seven urease gene subunit transcripts segments from the *C. sputorum* biovar *paraureolyticus* strain based on the sequence information (AB479194). RT-PCR mixtures contained 1×Qiagen OneStep RT-PCR Buffer, 80 µmol/L each dNTP, 60 µmol/L each primer and 20 ng mRNA.

Table 2. Urease activities of recombinant full-length urease genes cluster and seven 100% deletion recombinant variants of urease gene subunits from *C. sputorum* biovar *paraureolyticus* in transformed and log-phase cultured *E. coli* JM109 cells.

Recombinant variant	Urease activity (μmol/min/mg protein)	% value to full-length recombinant urease activity
C. sputorum biovar *paraureolyticus* lysate	2.765	146.63
E. coli cell lysate with recombinant full-length *C. sputorum* urease genes cluster	1.885	100.00
E. coli cell lysate with pGEM-T vector only	0.204	10.81
E. coli cell lysate with six *C. sputorum* urease genes (*ureG*)	0.193	10.25
E. coli cell lysate with six *C. sputorum* urease genes (*ureH*)	0.000	0.00
E. coli cell lysate with six *C. sputorum* urease genes (*ureA*)	0.000	0.00
E. coli cell lysate with six *C. sputorum* urease genes (*ureB*)	0.176	9.33
E. coli cell lysate with six *C. sputorum* urease genes (*ureC*)	0.197	10.44
E. coli cell lysate with six *C. sputorum* urease genes (*ureE*)	0.000	0.00
E. coli cell lysate with six *C. sputorum* urease genes (*ureF*)	0.000	0.00

RT-PCR began with Hot Start *Thermus aquaticus* (*Taq*) polymerase at 95°C for 5 min, followed by reverse transcription at 55°C for 30 min. The RT-PCR was carried out with 30 cycles at 94°C for 0.5 min, 50°C for 0.5 min, 72°C for 1 min, followed by a final extension at 72°C for 2 min in 10 μL reaction volumes using a Qiagen OneStep RT-PCR Kit (Qiagen). Amplified RT-PCR products were then separated by 1% (w/v) agarose gel electrophoresis in 0.5×TBE at 100 V and detected by staining with ethidium bromide.

Urease activities of the recombinant full-length *C. sputorum* biovar *paraureolyticus* urease genes and seven 100% deletion recombinant variants of urease gene subunits, each six urease genes containing -*ureG*, -*ureH*, -*ureA*, -*ureB*, -*ureC*, -*ureE* and -*ureF* constructed *in vitro*, were determined with log-phase cultured *E. coli* JM109 cells transformed with pGEM-T vector carrying the TA-cloned recombinant full-length *C. sputorum* urease gene cluster and seven 100% deletion recombinant variants. Recombinant *E. coli* JM109 variants cells were cultured in L Broth medium with or without addition of $NiCl_2$ (750 μmol/L) at 37°C.[25]

The *E. coli* JM109 cells carrying the recombinant *C. sputorum* urease gene cluster were pelleted by centrifugation (7000 ×*g*) at 4°C for 20 min. The cells were washed with phosphate-buffered saline (PBS) and resuspended in the same buffer. After the cells were disrupted by sonication, cell lysate extracts were collected by centrifugation (27,000 ×*g*) at 4°C for 30 min. Protein concentration determination of the extracts was conducted using the DC Protein Assay Kit (Bio-Rad Laboratories, Tokyo, Japan). Quantitative detection of urease activities was performed using the indophenol method (μmol/min/mg protein),[26] following the urease reaction of the fresh extract, by adding it to 50 mmol/L urea in PBS at 37°C for 4 h. In addition, the effects of $NiCl_2$ (750 μmol/L) in the *E. coli* culture medium on the urease activities were examined, as urease activities of the full-length recombinant UPTC CF89-12 urease gene cluster and the recombinant UPTC urease genes variants of the large (63% segment-deficient) and 100% *ureE* deletion have already been affected by $NiCl_2$, when cultured in the medium containing $NiCl_2$.[22]

In addition, the authors also examined the effects of $LiCl_2$ (10 mmol/L) in the *E. coli* medium on urease activity, as $LiCl_2$ has already been reported to accelerate urease activity in *H. pylori*.[26,27]

Finally, the effect of a urease inhibitor, thiourea (20 mmol/L), on the urease activity of the transformed and log-phase cultured *E. coli* JM109 cells carrying recombinant full-length *C. sputorum* biovar *paraureolyticus* urease gene cluster was examined,[28] in order to clarify whether or not the urease activities of the recombinant variants constructed in the present study are affected generally by urease inhibitor.

Results and discussion

In the present study, the authors first attempted to construct *in vitro* a recombinant full-length urease gene cluster from the *C. sputorum* biovar *paraureolyticus* LMG17591 strain, represented schematically in Figure 1, and to express the recombinant urease in the *E. coli* JM109 cells. As shown in Table 2, the recombinant *C. sputorum* biovar *paraureolyticus* urease gene cluster, including the TA-cloned full-length three structural (*ureA*, *ureB* and *ureC*) and four accessory (*ureG*, *ureH*, *ureE* and *ureF*) genes expressed catalytically active urease in *E. coli* cells (1.885 μmol/min/mg protein; Table 2).

Thus, urease enzyme activity of the recombinant full-length molecule of *C. sputorum* biovar *paraureolyticus* LMG17591 urease gene cluster was detected in the transformed and log-phase cultured *E. coli* JM109 cells using the indophenol method. In addition, almost no activity was detected in the cultured *E. coli* cells carrying only the pGEM-T vector (0.204 μmol/min/mg protein; Table 2).

As, in a previous study, the urease enzyme activity of the UPTC *ureE* large deletion recombinant variant (63% deficient of the full-length UreE subunit) showed an accelerated activity to approximately 15-fold and the activity in the 100% *ureE* deletion recombinant variant constructed also showed a positive acceleration (approximately 18-fold),[22] it is interesting to clarify whether or not this accelerated urease activity is detected in the transformed and log-phase cultured *E. coli* recombinant variants of seven 100% deletion *C. sputorum* biovar *paraureolyticus*.

Therefore, the authors constructed seven 100% deletion variants of the *C. sputorum* urease gene subunits in the *E. coli* JM109 cells, as shown schematically in Figure 1. No activities were detected carrying the six recombinant urease genes cluster (*ureG* through to *ureF*) (0.000–0.197 μmol/min/mg

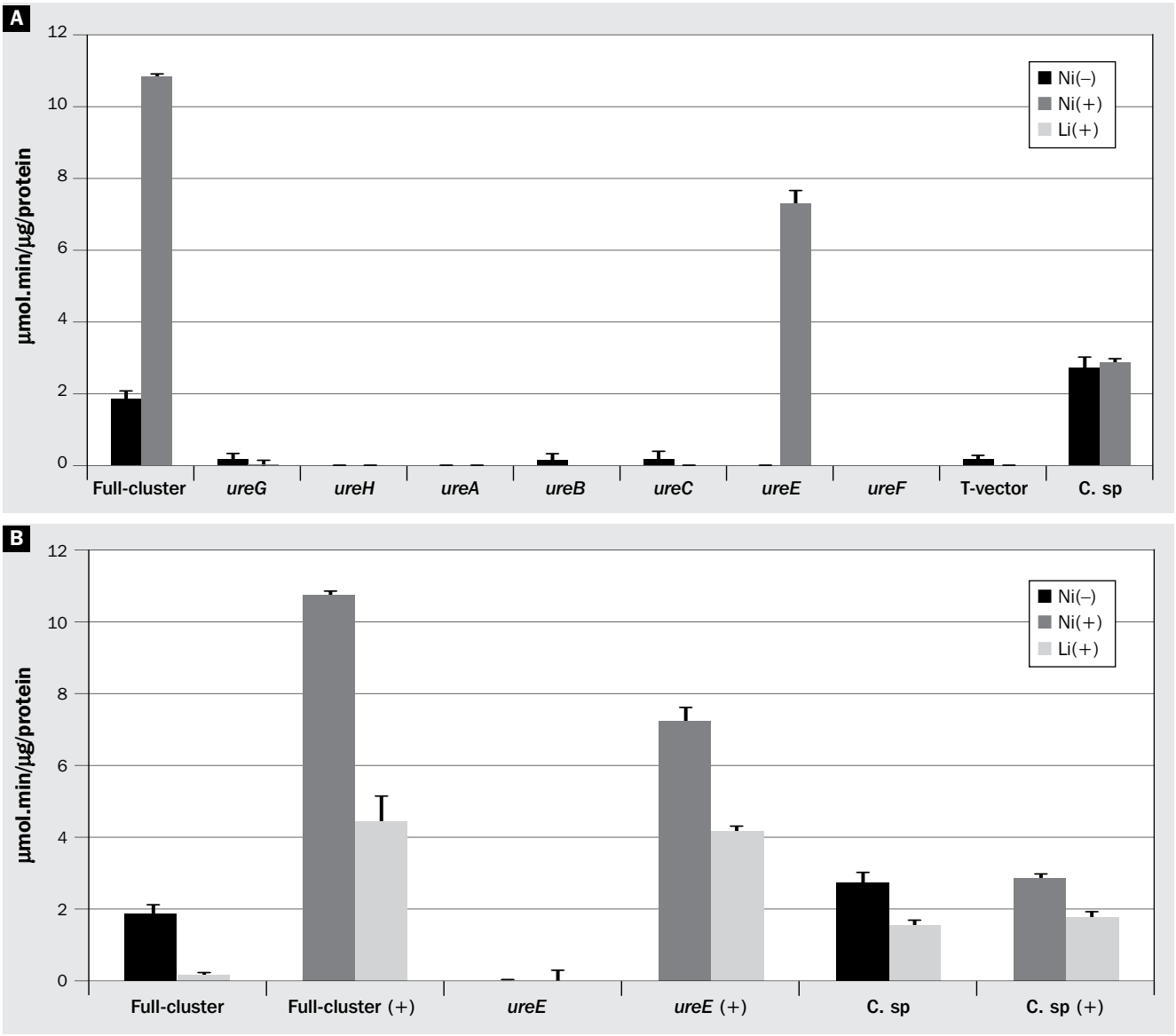


Fig. 3. Urease activity comparisons of *C. sputorum* biovar *paraureolyticus* among the preparations cultured in the medium containing $NiCl_2$ (750 µmol/L) or not containing $NiCl_2$ (A), and the effects of $LiCl_2$ (10 mmol/L) (B). Full-cluster, log-phase cultured *E. coli* JM109 cells carrying recombinant full-length urease gene cluster from the *C. sputorum* biovar *paraureolyticus* LMG17591 strain (*ureG*, *ureH*, *ureA*, *ureB*, *ureC*, *ureE* and *ureF*), transformed and log-phase cultured *E. coli* cells carrying seven 100% deletion recombinant variants of the urease gene subunits of *C. sputorum*; T-vector, transformed and log-phase cultured *E. coli* cells transformed with pGEM-T Vector only. C. sp.: *C. sputorum* biovar *paraureolyticus* LMG17591 cell lysate.

protein; Table 2). Consequently, among seven 100% deletion recombinant variants of the *C. sputorum* urease each subunit genes, all 100% deletion variants showed no change in the assay of the urease reaction, as in the *E. coli* cell lysate with pGEM-T vector only as a control (Table 2). In addition, the *ureE* deletion recombinant variant showed no positive and accelerated urease activity (Table 2).

Thus, in the present study with the seven 100% deletion recombinant variants of the urease each gene subunits from *C. sputorum*, no accelerated urease enzyme activity was identified in the 100% *ureE*-deletion recombinant variant, in contrast to the UPTC CF89-12 strain, as previously shown by Nakanishi *et al*.

The RT-PCR analyses were carried out using the seven primer pairs, f-/r-CspureG, f-/r-CspureH, f-/r-CspureA, f-/r-CspureB, f-/r-CspureC, f-/r-CspureE and f-/r-CspureF, in order to clarify whether or not each from the seven 100% deletion recombinant variants of the urease gene subunits from *C. sputorum* biovar *paraureolyticus* (*ureG* through to *ureF*) could be transcribed in the *E. coli* cells (Figs. 2A and B). Figure 2C showed that no corresponding gene of the seven recombinant subunit genes were expressed at the transcriptional level in the 100% deletion recombinants variants (*ureG* through to *ureF* in Fig. 2C). In addition, the other six remaining urease subunits genes were all transcribed within the seven 100% deletion recombinant variants (Fig. 2C).

Thus, the present RT-PCR analyses confirmed the successful construction of seven 100% deletion recombinant variants of each of the urease gene subunits from the *C. sputorum* biovar *paraureolyticus* in *E. coli* JM109 cells.

Effects of $NiCl_2$ (750 µmol/L) were also examined in the

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Csp UreE    1 MIVNKILGNLKDYDTKHKTIAWVNIHPGDRLKKIVRLKADN--GNEFGINLDDDVILKDG  58
Cl UreE     1 ..--LLQNKI.H..L-N.ECDFLELSWF.TF...L.TTTLK--.LDVA.KMP.NKG.NHN  55
Ka UreE     1 .L-----YLTQRLEIPAAAT.S.TLPI-.VRV.S-.V.VTLND.RDA.LL.PRGLL.RG.  53
              *                            *   *  *       *            *

Csp UreE   59 DVL-GEDDENIF-VLKCFPQN-VIVIKPDTLT-------QMGFVAHSIGNN--HTPAIFE 106
Cl UreE    56 .C.YD..--FLIL.-.IK.EK-.LK.HIENEYNLALISY.V.NM-.LNLFYKD.KLLTL. 110
Ka UreE    54 ...SN.EGTEF--.QVIAADEE.S.VRC.DPFMLAKACYHL.NR-.VPLQIMPGELRYHH 110
              * *  *       *        *                  *   *

Csp UreE  107 -NDTMIVEYDYIIEKMLNEMKVSFERKDLVLDTPLKHASHHH---------- 147
Cl UreE   111 Q.SIIRFL-----..FNIKYEKCE.--I.EPKYM.DMP.FIQVDPNFKLIKE 155
Ka UreE   111 DHVLDDMLRQFGLTVTFGQLPFEP.AGAYASES---.-G...AHHDHHAHSH 158
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Fig. 4. Deduced amino acid sequence alignment analysis of the possible ORFs of the full-length *C. sputorum ureE* (Csp UreE), as well as possible ORFs of the full-length urease gene *ureE* from UPTC CF89-12 (Cl UreE) and *K. aerogenes* (Ka UreE).

transformed and log-phase cultured *E. coli* cell culture medium on the urease activity of the full-length recombinant *C. sputorum* biovar *paraureolyticus* LMG17591 urease gene cluster and the recombinant *C. sputorum* biovar *paraureolyticus* urease gene variants of the 100% *ureE* deletion. These urease activities were positively affected by $NiCl_2$, as shown in Figure 3A (full-cluster and *ureE*) and Table 3. Consequently, the recombinant full-length urease gene cluster and 100% deletion recombinant of the *ureE* gene from the *C. sputorum* biovar *paraureolyticus* in the transformed and log-phase cultured *E. coli* cells showed positively accelerated urease activities, when cultured in the medium containing the $NiCl_2$ (Fig. 3A and Table 3). In addition, no urease activity from the *C. sputorum* biovar *paraureolyticus* cell lysates appear to be affected by $NiCl_2$ (750 µmol/L). However, more attempts may be needed in order to clarify the effect of $NiCl_2$ on the *C. sputorum* urease activity, as, in the present study, the *C. sputorum* cells was cultured in Mueller-Hinton broth containing $NiCl_2$ (750 µmol/L), and a nickel-containing liquid culture was employed for the *E. coli* cells.

In the present study, effects of the $LiCl_2$ (10 mmol/L) on the *C. sputorum* and recombinant *C. sputorum* urease activities were examined (Fig. 3B). When $LiCl_2$ was added to the full-cluster, log-phase cultured *E. coli* cells carrying the recombinant molecule of the full-length urease gene cluster from the *C. sputorum* strain with or without $NiCl_2$, *ureE* log-phase cultured *E. coli* cells carrying the *ureE* 100% deletion recombinant variant of the urease gene cluster of the *C. sputorum* with or without $NiCl_2$, and *C. sputorum* biovar *paraureolyticus* LMG17591 cell lysate with or without $NiCl_2$, the urease activities were suppressed in all six cases (Fig. 3B). Thus, $LiCl_2$ (10 mmol/L) exhibited an inhibitory effect on the six *C. sputorum* urease activities.

Previously, biochemical characteristics of the purified UPTC CF89-12 strain urease (i.e., pH activity, stability against heat, effect of inhibitors on urease activity) have been clarified; hydroxyurea, thiourea, *N*-ethylmaleimide and acetohydroxamic acid inhibited UPTC urease by 50% at 7.0 mmol/L, 15.0 mmol/L, 4.9 mmol/L and 3.0 mmol/L, respectively.[30] In the present study, thiourea (20 mmol/L) completely inhibited urease activity from all examples, including the recombinant variants of the *C. sputorum* biovar *paraureolyticus* examined (data not shown).

Recombinant full-length *C. sputorum* biovar *paraureolyticus* urease seven-gene cluster, three structural *ureA*, *ureB* and *ureC* and four accessory *ureE*, *ureF*, *ureG* and *ureH* genes, constructed including promoter and terminator structures, expressed a urease enzyme activity in the transformed and log-phase cultured *E. coli* cells. As described previously,[23] some accessory genes, such as *ureI* other than the four *ureE*, *ureF*, *ureG* and *ureH(D)* genes, were undetectable in the *C. sputorum* biovar *paraureolyticus* LMG17591 urease gene cluster operon. In addition, the *ureE*-deletion recombinant variant showed no urease-positive reaction, in contrast with UPTC CF89-12, only *ureE*-large deletion (63% deficient) and *ureE*-complete deletion (100% deficient) recombinant variants showed an accelerated positive reaction (approximately 15-fold and 18-fold, respectively), among the several deletion recombinant variants (*ureA*-, *ureB*-, *ureE*-, *ureF*-, *ureG*- and *ureH*- large deficient).[22]

Regarding UreE, *Klebsiella aerogenes* UreE contains a histidine-rich carboxyl terminus sequence and is able to bind five to six Ni^{2+} ions per dimer.[29,30] In a previous study, UPTC *ureE* and its deduced amino acid sequence of the possible open reading frame (ORF) from the UPTC *ureE* sequenced and analysed, was shown to lack the histidine-rich carboxyl terminus (Fig. 4). In the present study, a small histidine-rich

Table 3. $NiCl_2$ (750 µmol/L) effects on urease activities in recombinant full-length *C. sputorum* biovar *paraureolyticus* urease gene cluster.

Recombinant variant	µmol/min/mg protein	% activity of wild type
C. sputorum biovar *paraureolyticus* lysate	2.902	153.95
C. sputorum urease gene cluster *E. coli* cell lysate with the recombinant full-length	10.877	577.03
C. sputorum urease gene (*ureE*) *E. coli* cell lysate with the recombinant variant	7.336	390.83

carboxyl terminus (4/6) occurred in the possible ORF of the full-length *C. sputorum* biovar *paraureolyticus ureE* (Fig. 4). In addition, only 36 identical amino acid residues existed between the possible ORFs of the Csp UreE (147 amino acid residues) and the Cl UreE (155 residues) (Fig. 4).

This is the first demonstration of construction, expression and characterisation of recombinant full-length urease gene cluster and seven 100% deletion recombinant variants of urease gene subunits from the *C. sputorum* biovar *paraureolyticus* LMG17591 strain with transformed and log-phase cultured *E. coli* JM109 cells.

In addition to the expression of the urease activity in *E. coli* cells harbouring the full-length recombinant *C. sputorum* biovar *paraureolyticus* urease gene cluster, urease subunits A and C, which may be encoded on *ureA* and *ureC*, were also confirmed immunologically by Western blot analysis with anti-urease α (A) and β (B) raised against *H. pylori* (data not shown). This result strongly suggests that these two subunits are immunologically reactive between *C. sputorum* biovar *paraureolyticus* and *H. pylori*, as well as UPTC CF89-12, as described previously.[22]

When, in a previous study,[22] the authors carried out a quantitative detection of urease activity using the indophenol method (specific activity, μmol/min/mg protein) following urease reaction of fresh extract (4 μg) (from the UPTC CF89-12 cell lysate after adding to 50 mmol/L urea in PBS at 37°C for 4 h), a urease activity of 2.403 μmol/min/mg protein was detected. In addition, in the present study, *C. sputorum* biovar *paraureolyticus* LMG17591 cell lysate gave a urease activity determined to be 2.765 μmol/min/mg protein with a similar determination procedure, as employed for the UPTC urease described above.

The authors have already described a neighbour-joining tree constructed based on the nucleotide sequence information of urease genes showing that *C. sputorum* biovar *paraureolyticus* formed a cluster with *Arcobacter butzleri*, UPTC and some *Helicobacter* spp., separating those from the other urease-producing bacteria, suggesting a commonly shared ancestry among these organisms.[23] Regarding the urease activity from *H. pylori* isolates, Dunn *et al*. reported the specific activity of lysates from 15 *H. pylori* isolates varying from 3.3 to 25.5 μmol urea hydrolysed per min/mg of protein (mean: 7.8±5.6 μmol urea hydrolysed/min/mg protein).[31]

Thus, specific activity levels of urease from the *Campylobacter* organisms (2.403 and 2.765 μmol/min/mg protein), as described above, have been identified and are lower than those from *H. pylori* organisms. □

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